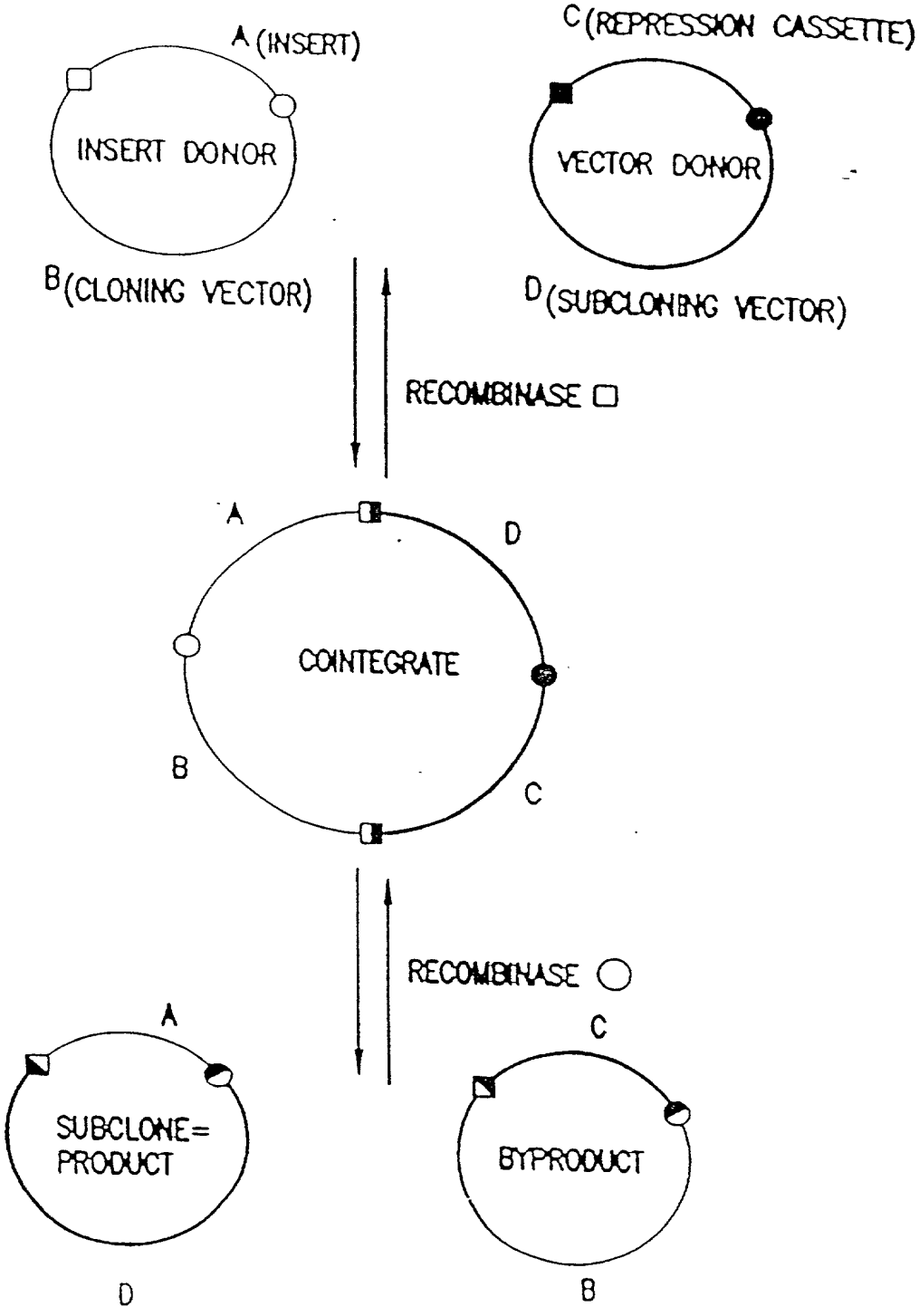

A (INSERT)
INSERT DONOR
B (CLONING VECTOR)
C (REPRESSION CASSETTE)
VECTOR DONOR
D (SUBCLONING VECTOR)
RECOMBINASE □
A
D
COINTEGRATE
B
C
RECOMBINASE ○
A
SUBCLONE=
PRODUCT
D
C
BYPRODUCT
B

FIG.1

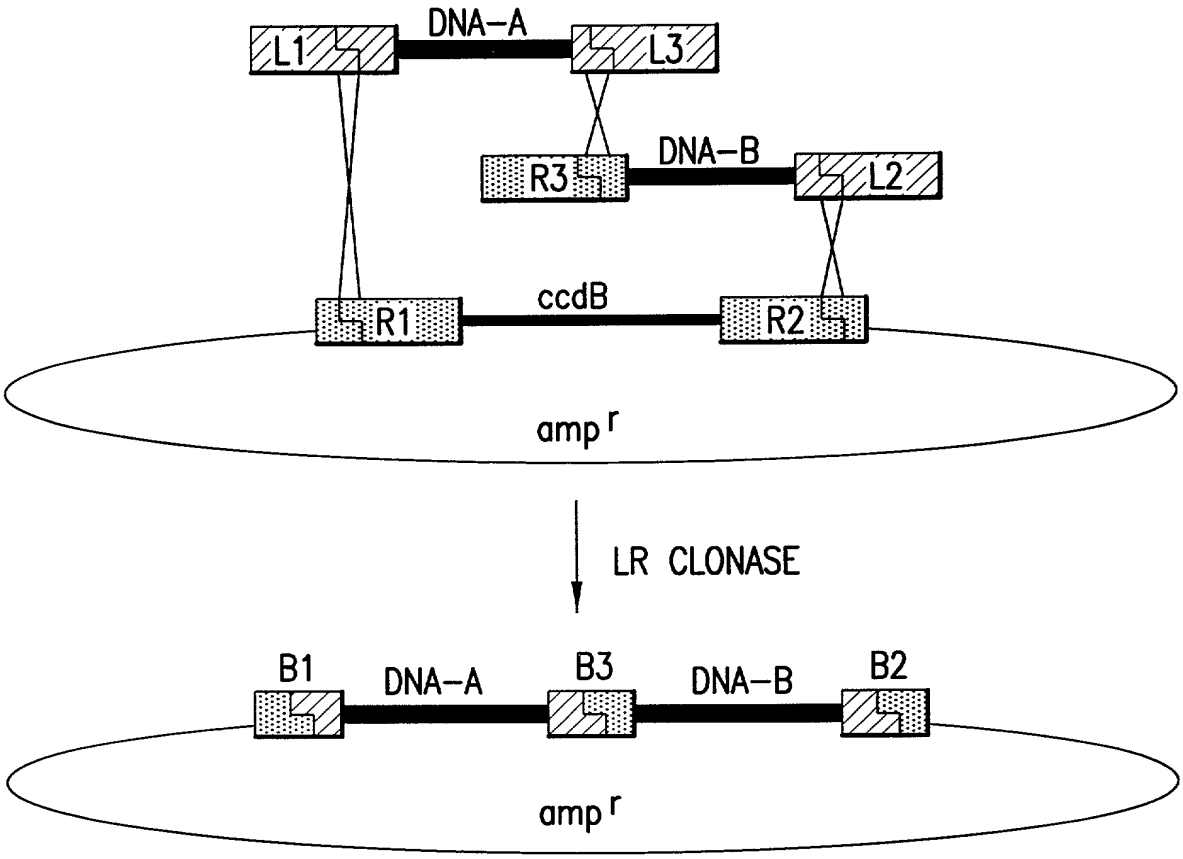

DNA–A
L1
L3
DNA–B
R3
L2
ccdB
R1
R2
amp r
LR CLONASE
B1
DNA–A
B3
DNA–B
B2
amp r

FIG.2

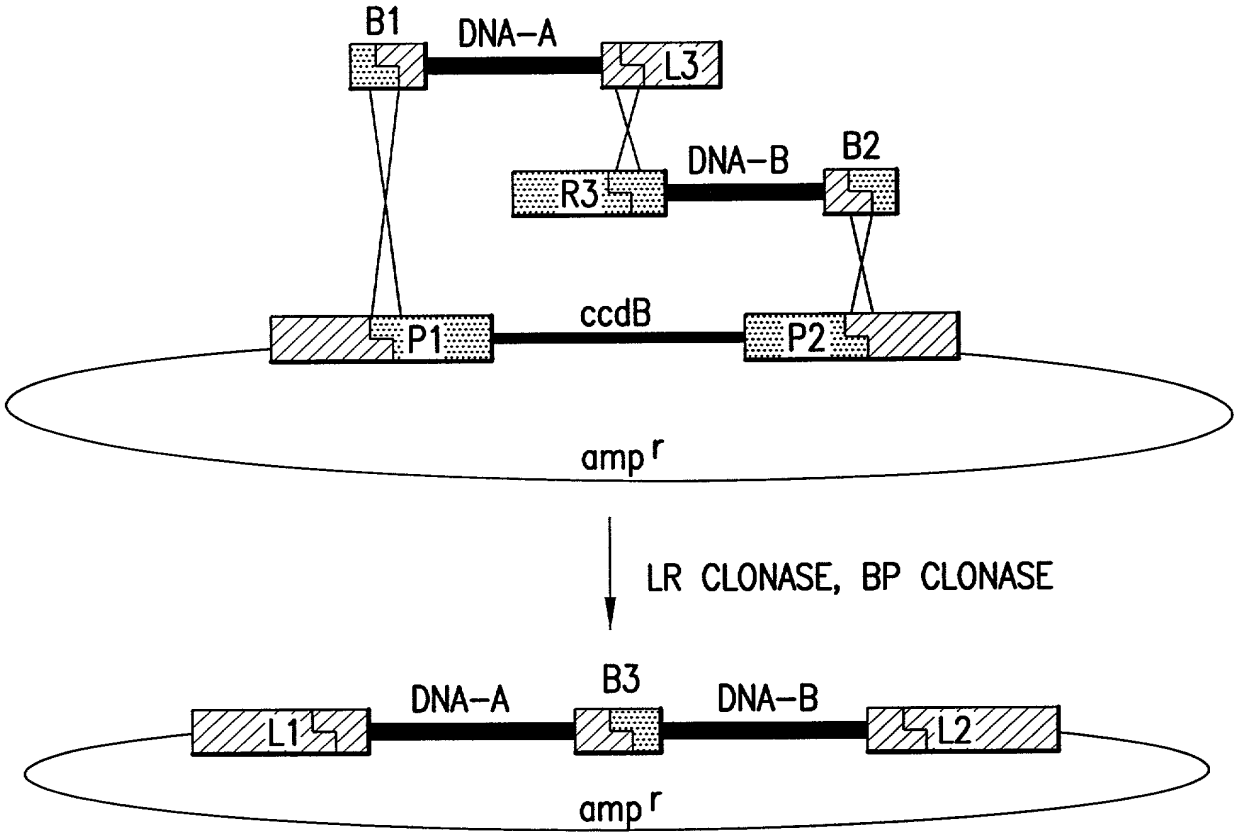

B1
DNA–A
L3
DNA–B
B2
R3
ccdB
P1
P2
amp r
LR CLONASE, BP CLONASE
DNA–A
B3
DNA–B
L1
L2
amp r

FIG.3

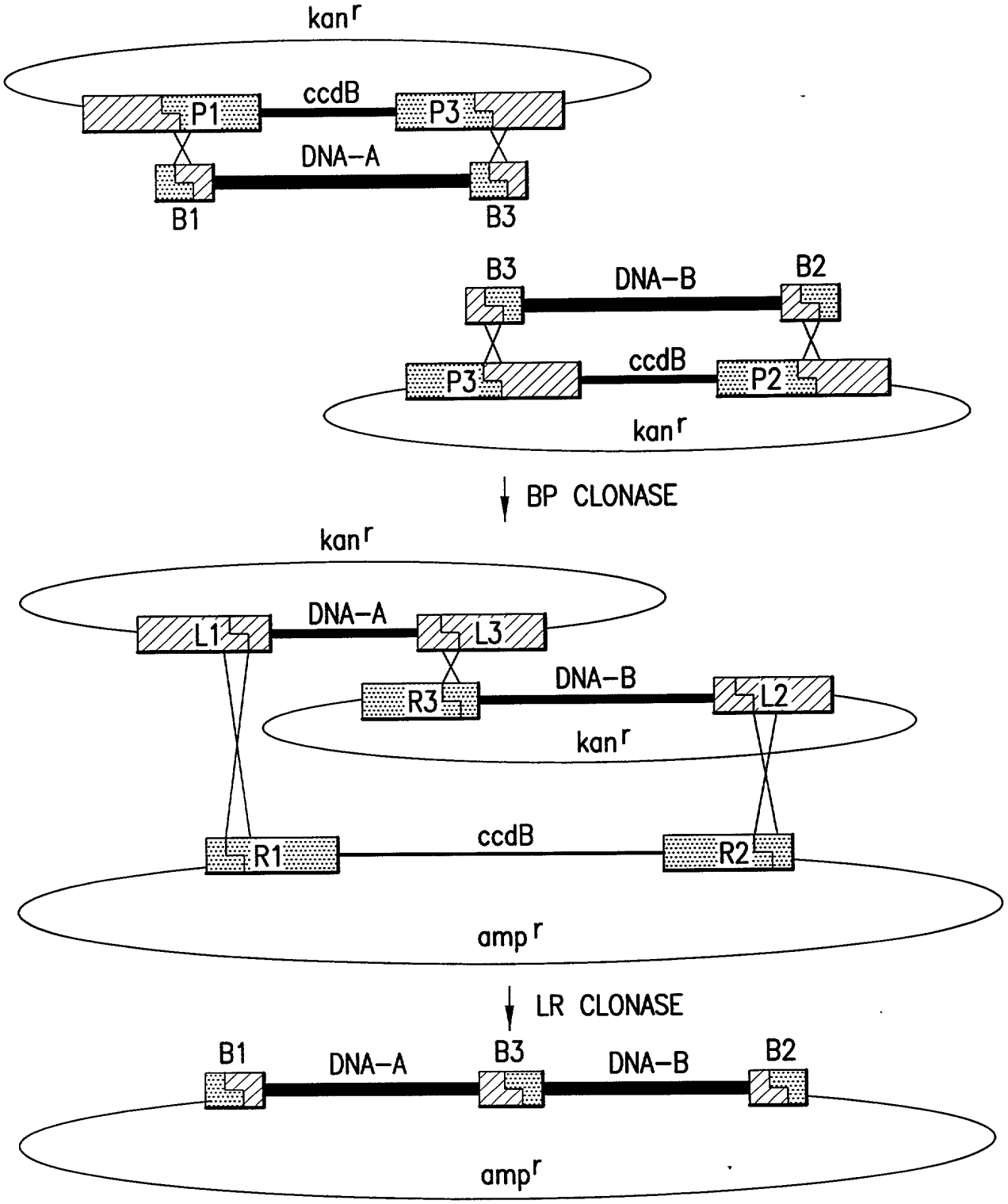

kan r
ccdB
P1
P3
DNA–A
B1
B3
B3
DNA–B
B2
ccdB
P3
P2
kan r
BP CLONASE
kan r
DNA–A
L1
L3
DNA–B
R3
L2
kan r
ccdB
R1
R2
amp r
LR CLONASE
B1
DNA–A
B3
DNA–B
B2
amp r

FIG.4

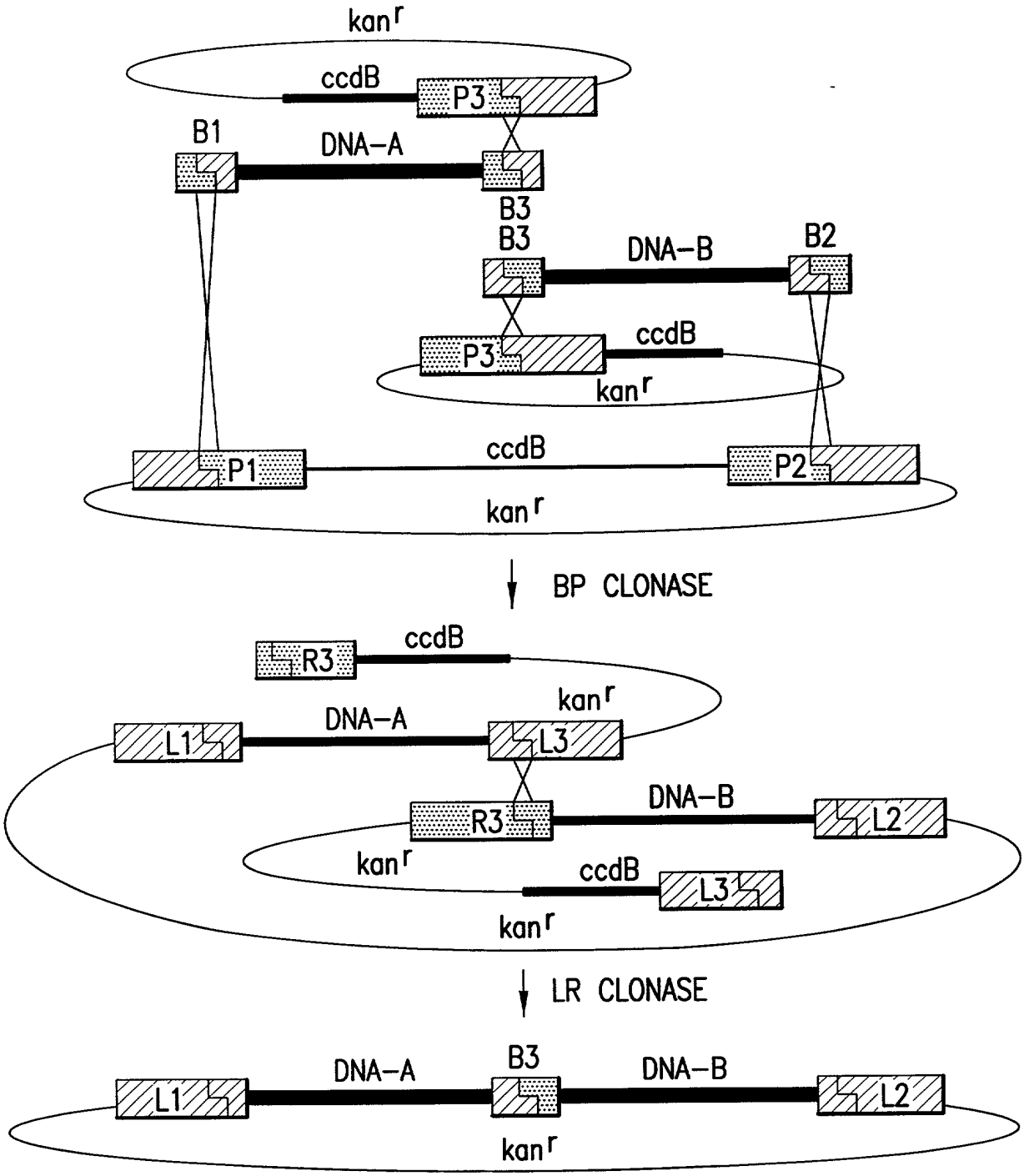

kan r
ccdB
P3
B1
DNA–A
B3
B3
DNA–B
B2
P3
ccdB
kan r
P1
ccdB
P2
kan r
BP CLONASE
R3
ccdB
kan r
L1
DNA–A
L3
R3
DNA–B
L2
kan r
ccdB
L3
kan r
LR CLONASE
L1
DNA–A
B3
DNA–B
L2
kan r

FIG.5

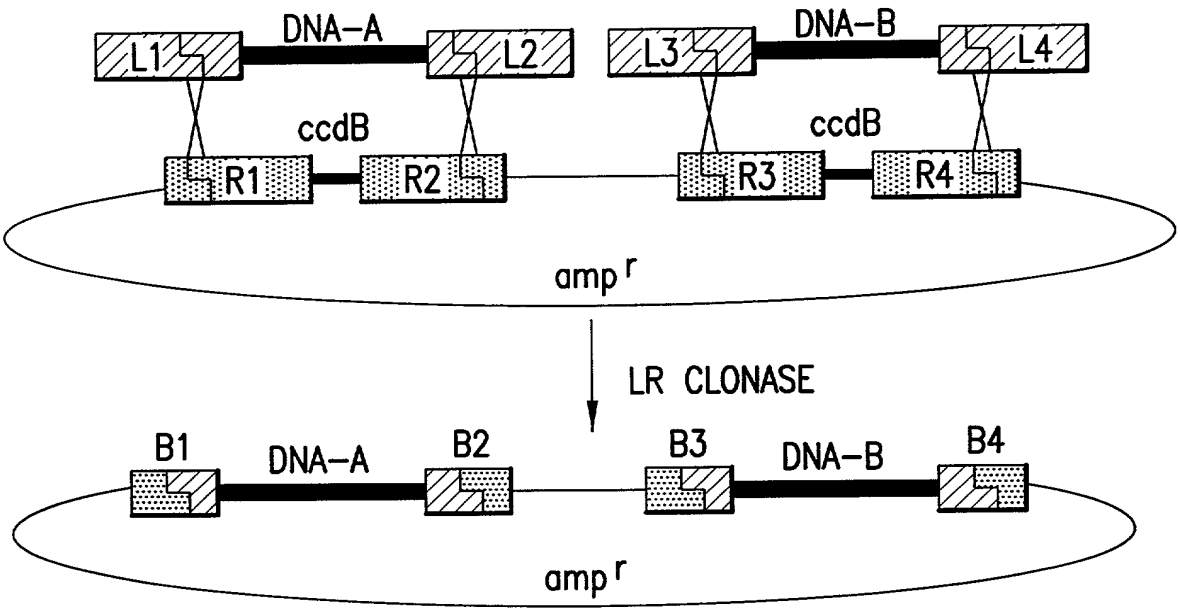

L1
DNA–A
L2
L3
DNA–B
L4
ccdB
ccdB
R1
R2
R3
R4
amp r
LR CLONASE
B1
DNA–A
B2
B3
DNA–B
B4
amp r

FIG.6

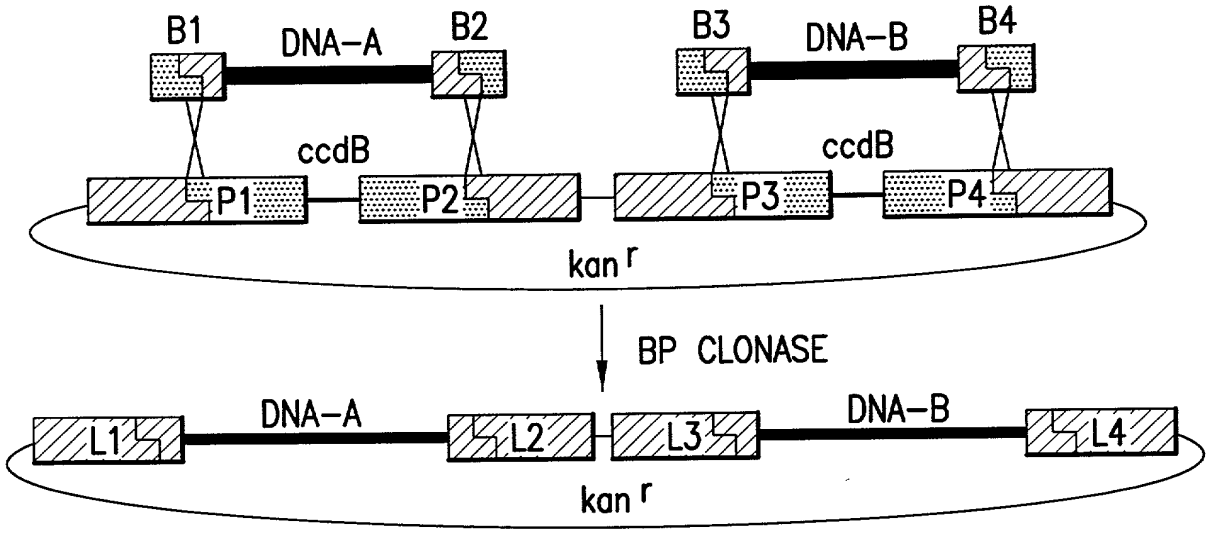

B1
DNA–A
B2
B3
DNA–B
B4
ccdB
ccdB
P1
P2
P3
P4
kan r
BP CLONASE
L1
DNA–A
L2
L3
DNA–B
L4
kan r

FIG.7

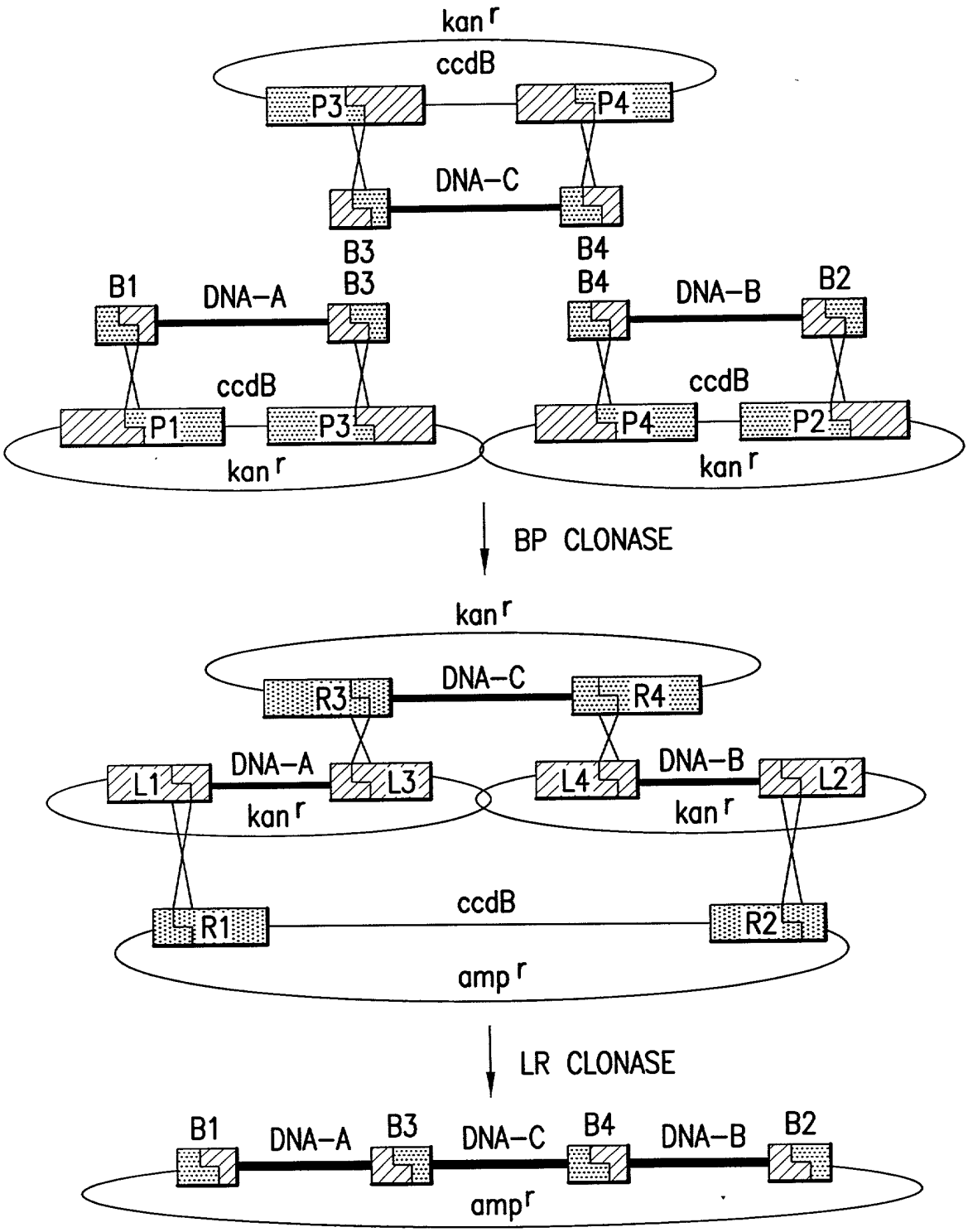

kan r
ccdB
P3
P4
DNA–C
B3
B4
B1
DNA–A
B3
B4
DNA–B
B2
ccdB
ccdB
P1
P3
P4
P2
kan r
kan r
BP CLONASE
kan r
DNA–C
R3
R4
DNA–A
DNA–B
L1
L3
L4
L2
kan r
kan r
ccdB
R1
R2
amp r
LR CLONASE
B1
DNA–A
B3
DNA–C
B4
DNA–B
B2
amp r

FIG.8

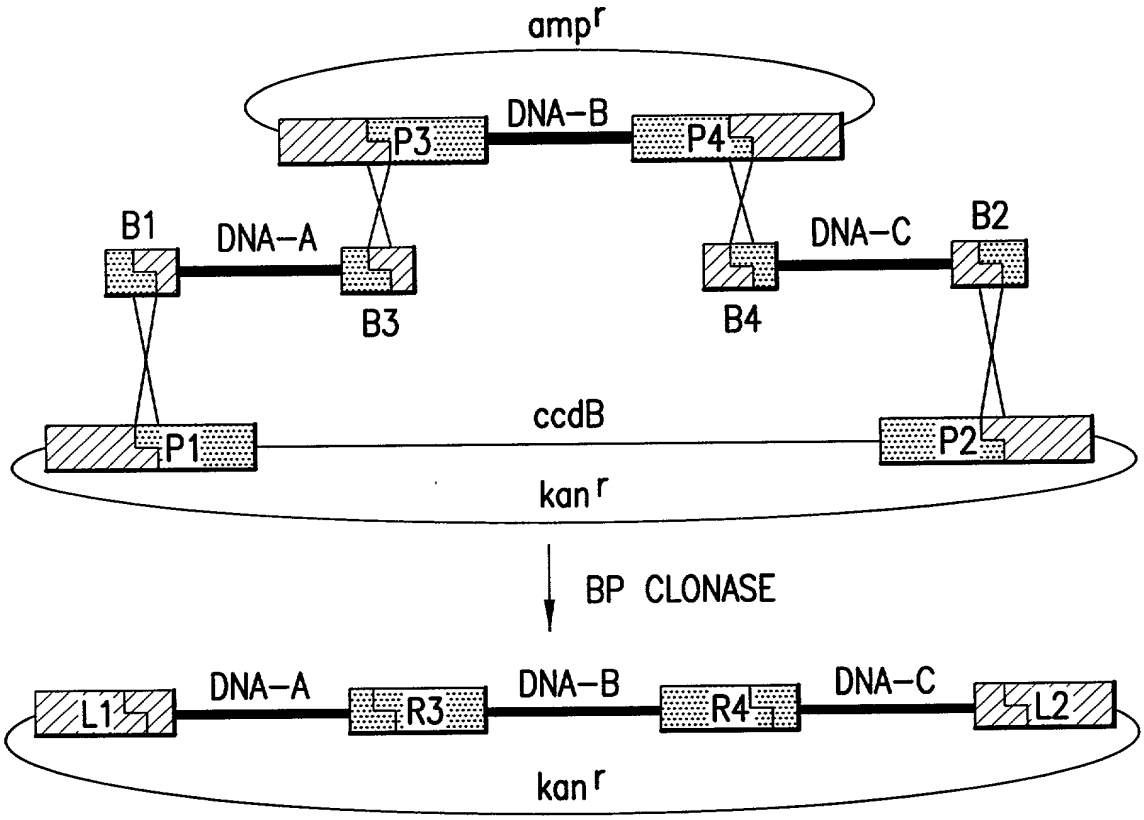

amp r
DNA–B
P3
P4
B1
DNA–A
B3
DNA–C
B2
B4
ccdB
P1
P2
kan r
BP CLONASE
L1
DNA–A
R3
DNA–B
R4
DNA–C
L2
kan r

FIG.9

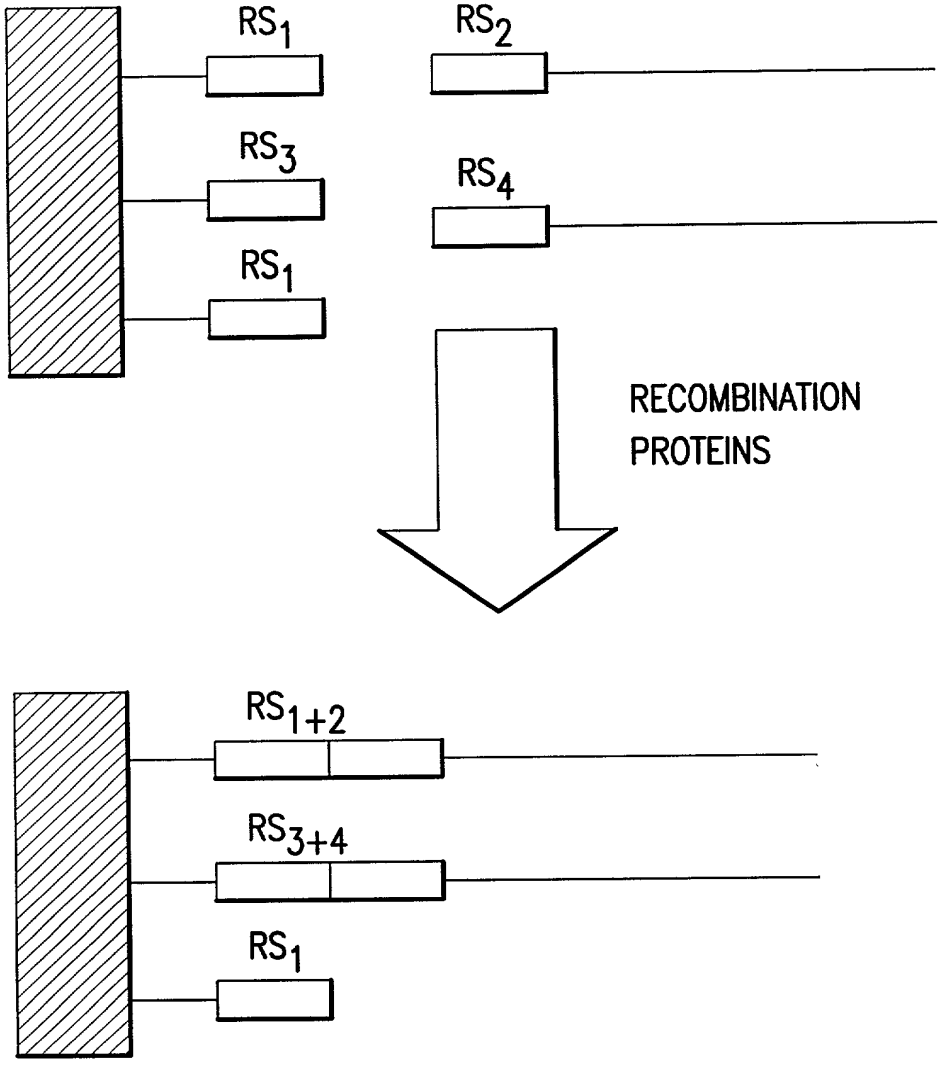


FIG.10

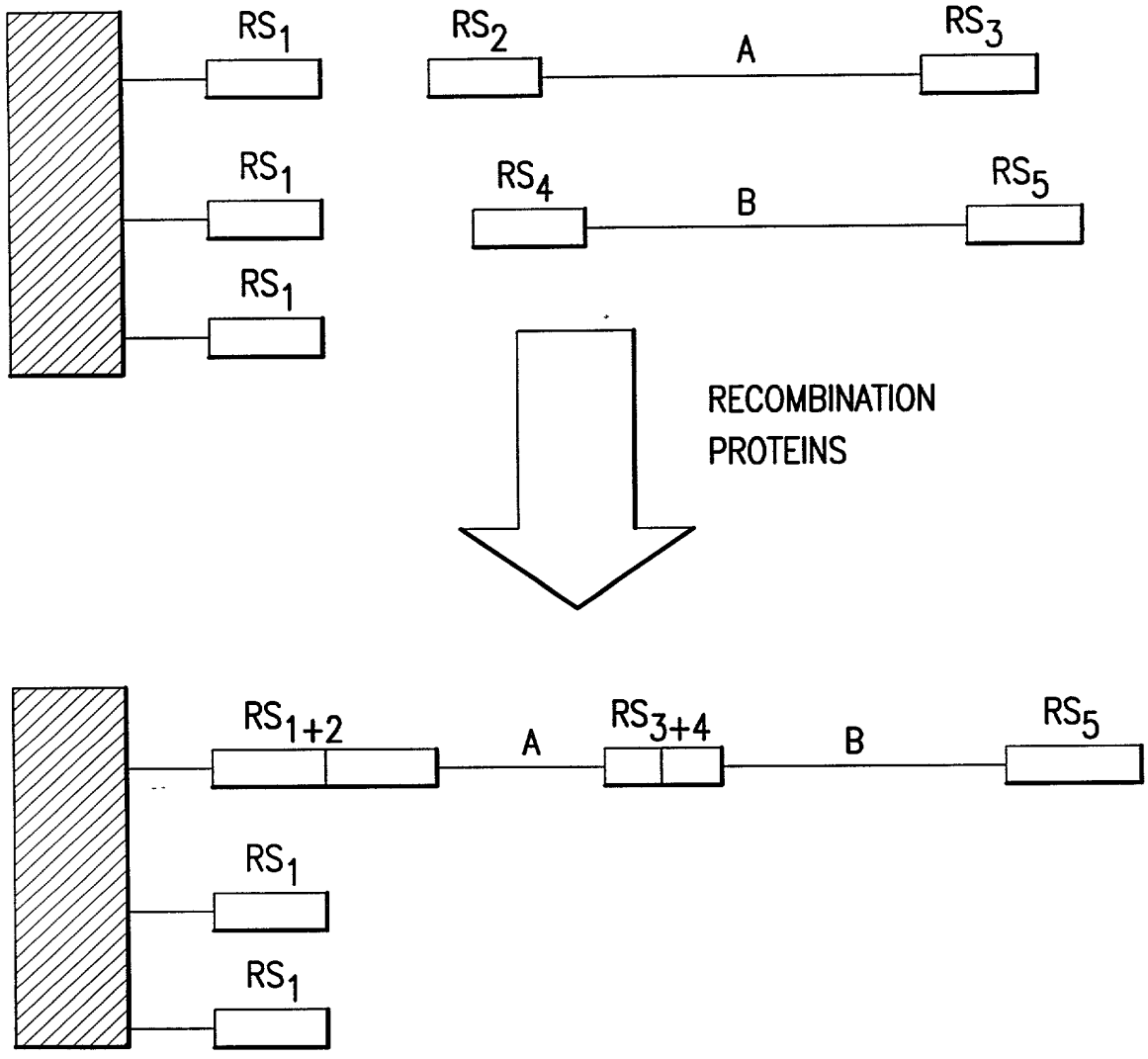

RS1
RS1
RS1
RS2
A
RS3
RS4
B
RS5
RECOMBINATION
PROTEINS
RS1+2
A
RS3+4
B
RS5
RS1
RS1

FIG.11

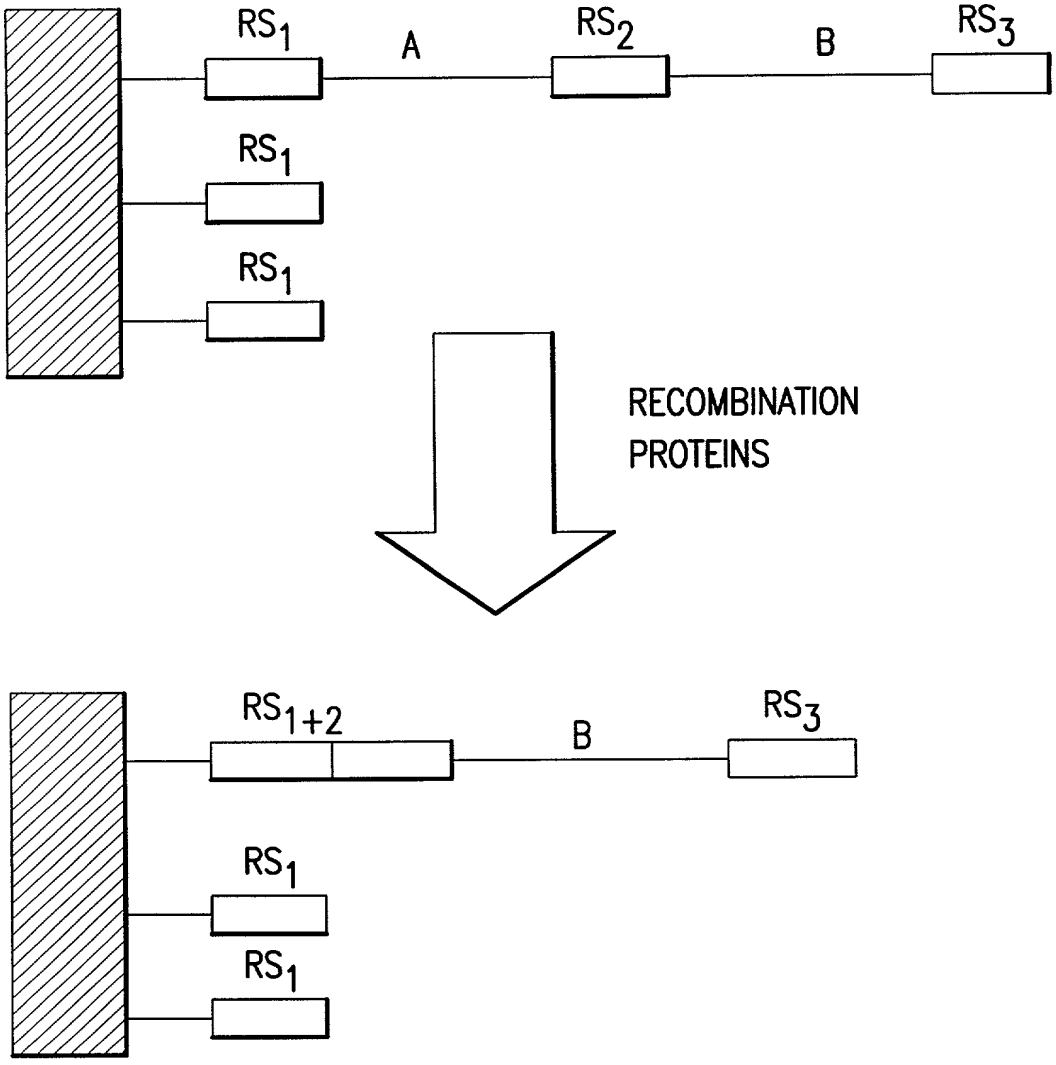

RS1
A
RS2
B
RS3
RS1
RS1
RECOMBINATION
PROTEINS
RS1+2
B
RS3
RS1
RS1

FIG.12

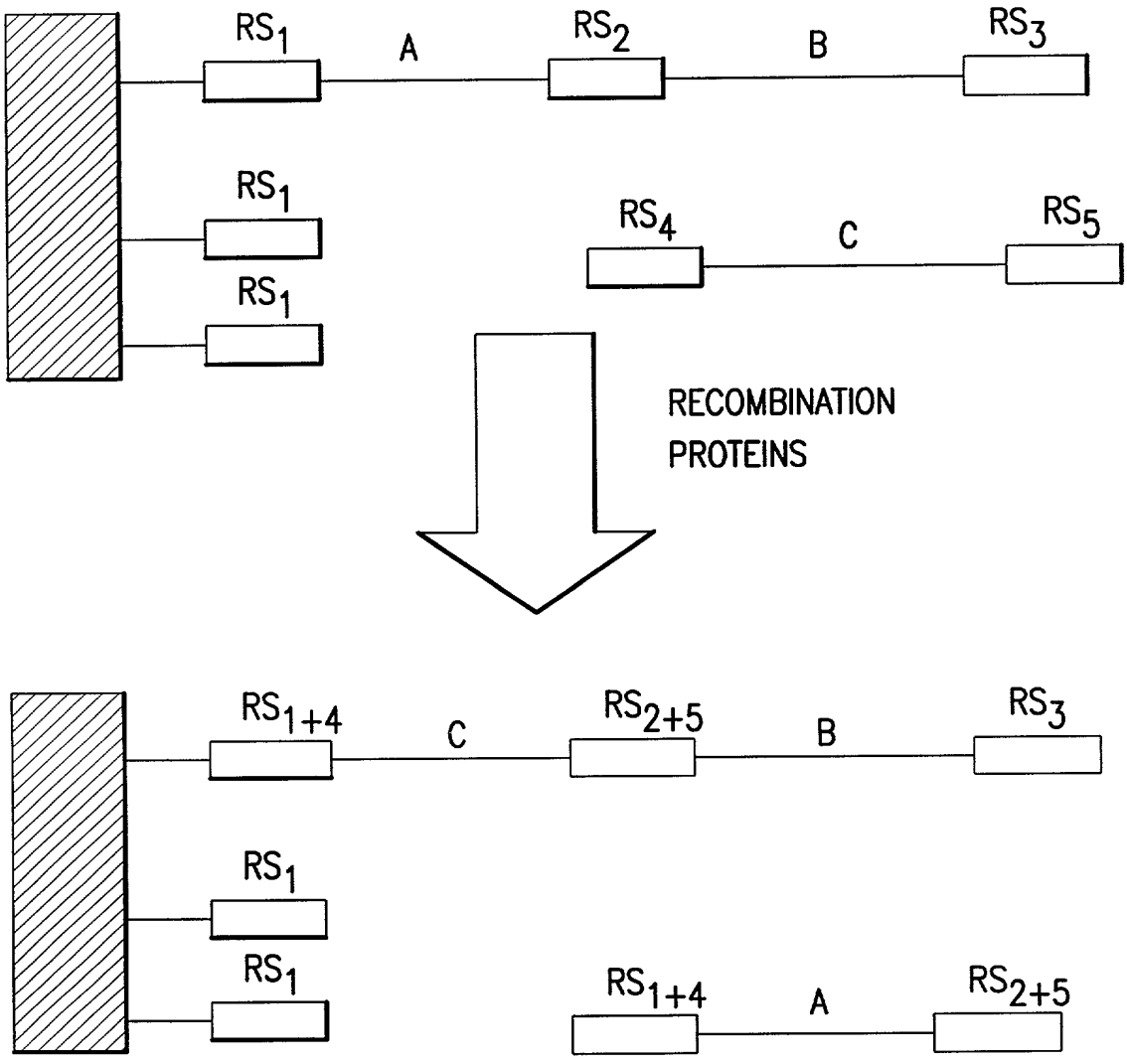

RS1
A
RS2
B
RS3
RS1
RS4
C
RS5
RS1
RECOMBINATION
PROTEINS
RS1+4
C
RS2+5
B
RS3
RS1
RS1
RS1+4
A
RS2+5

FIG.13

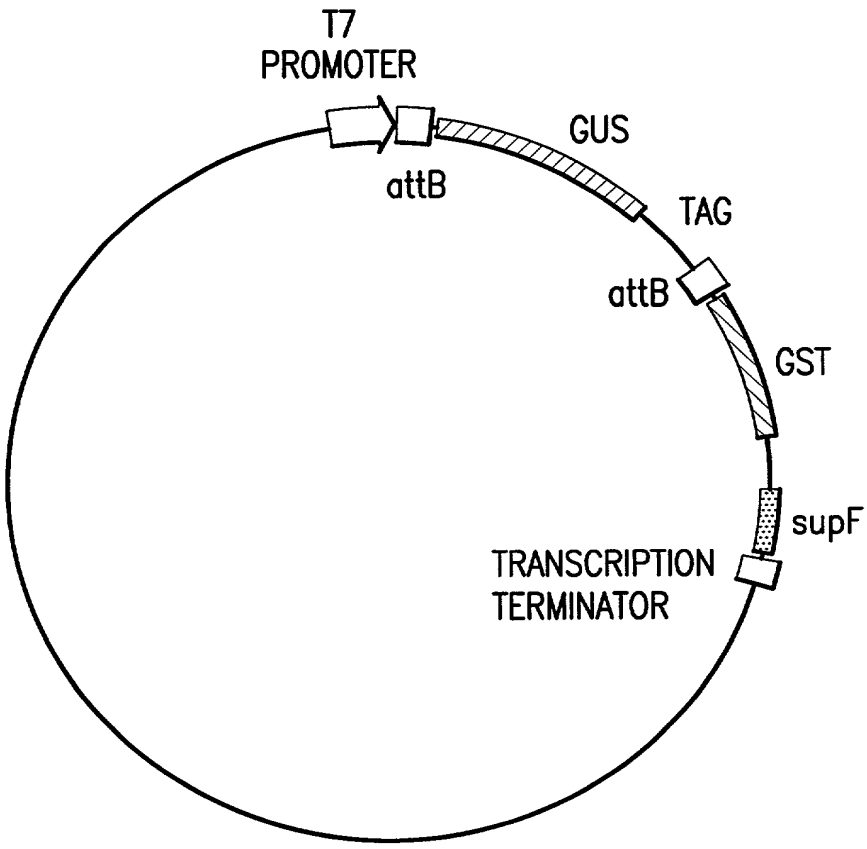

T7
PROMOTER
attB
GUS
TAG
attB
GST
supF
TRANSCRIPTION
TERMINATOR

FIG.14A

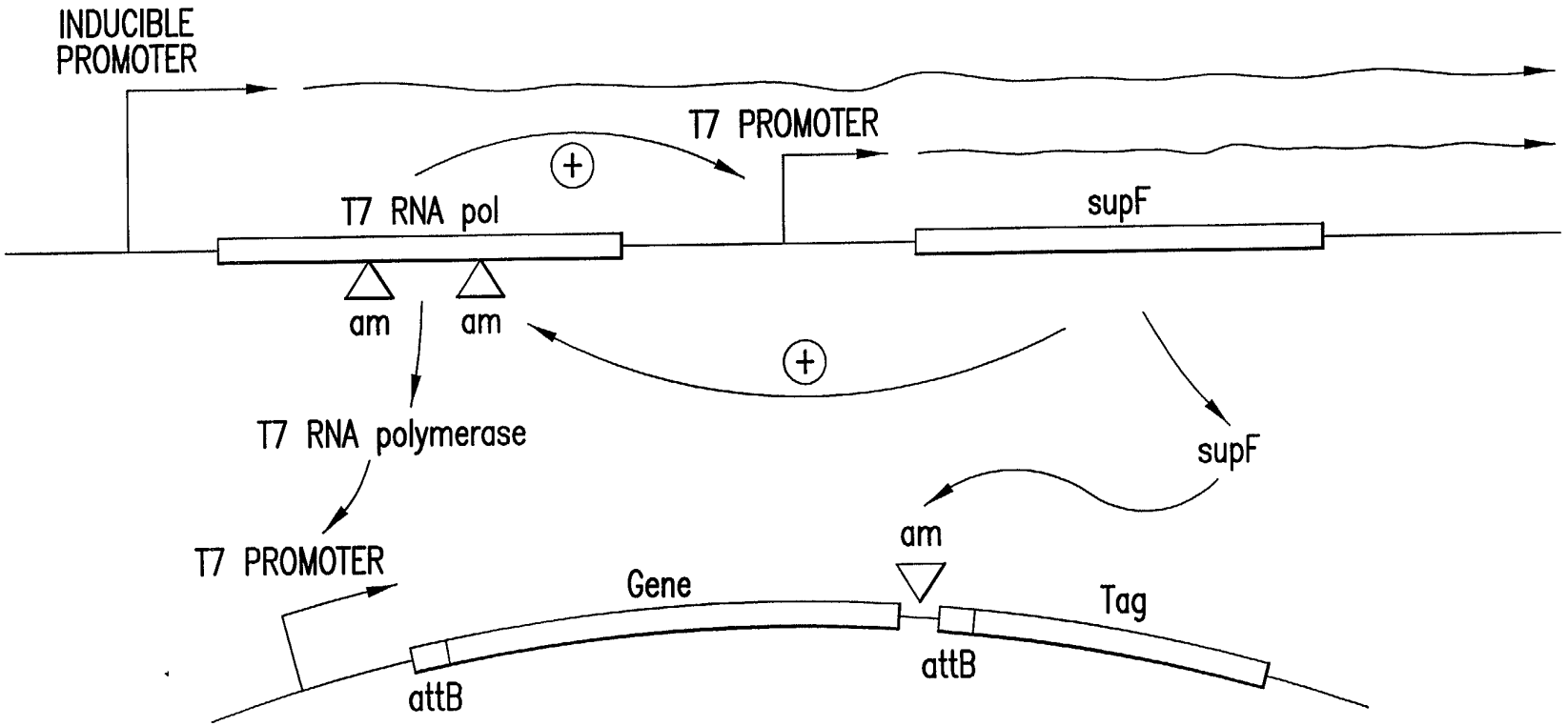

INDUCIBLE
PROMOTER
T7 PROMOTER
T7 RNA pol
supF
am
am
T7 RNA polymerase
supF
T7 PROMOTER
am
Gene
Tag
attB
attB

FIG.14B

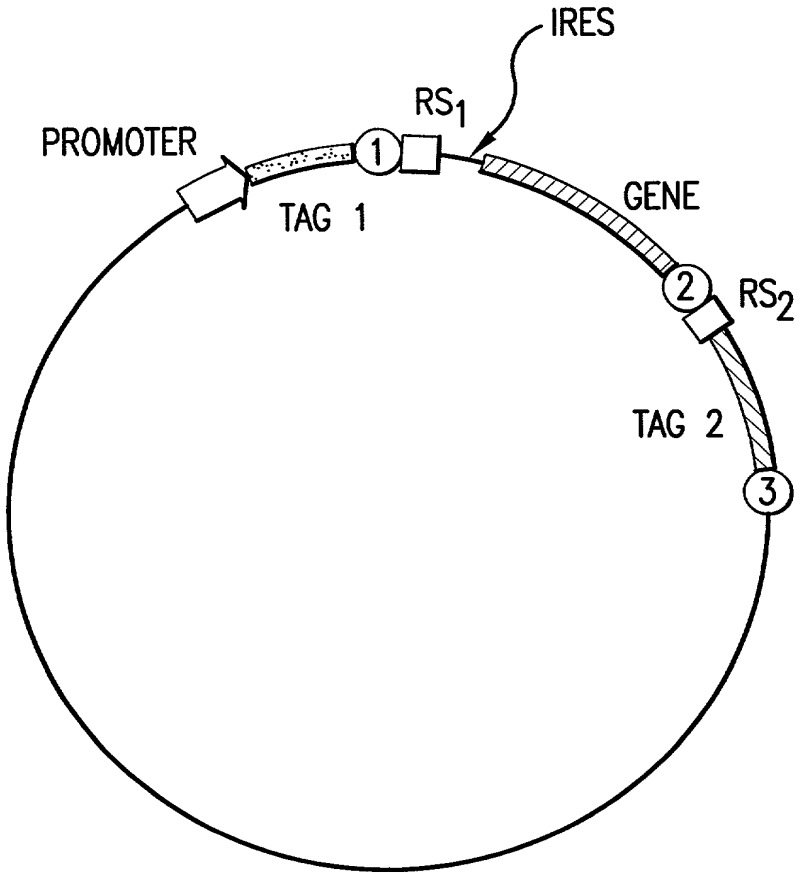


FIG.15

FIGURE 16

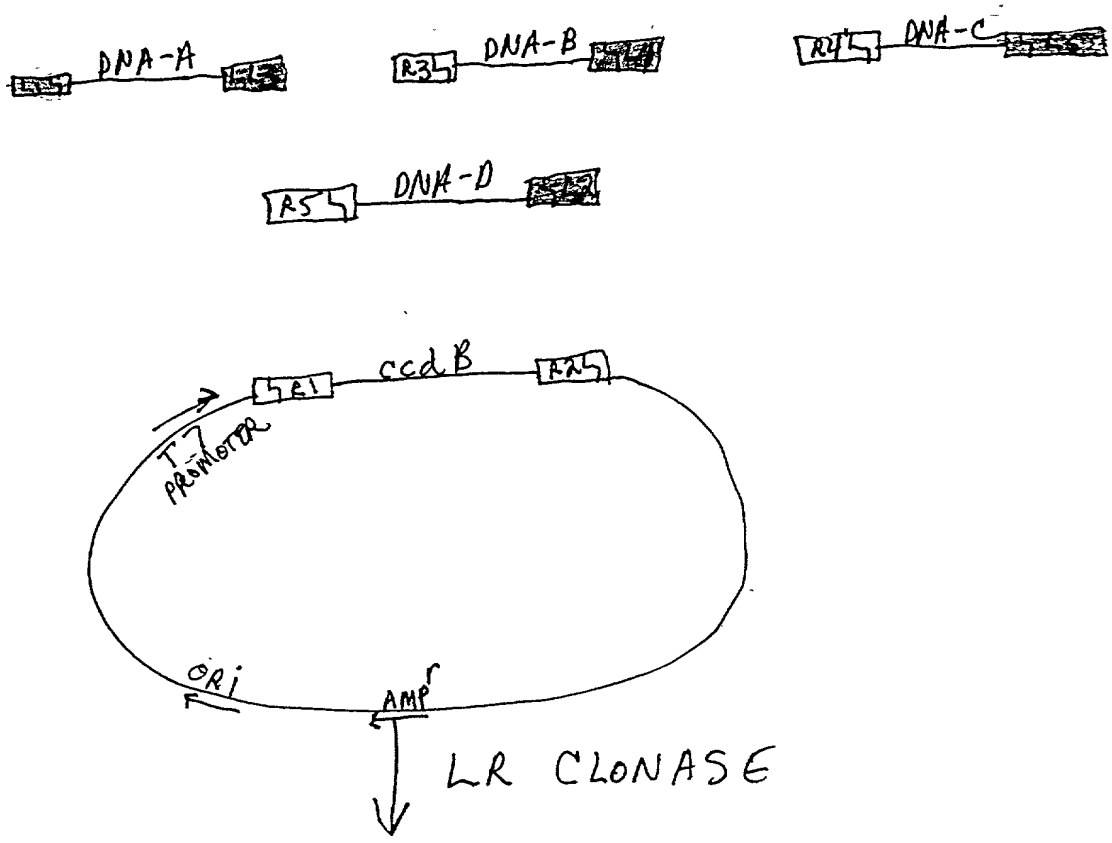

DNA-A
DNA-B
DNA-C
DNA-D
R1
R2
R3
R4
R5
ccdB
T7 PROMOTER
ORi
AMP^r
LR CLONASE
B1
B2
B3
B4
B5

FIGURE 17A

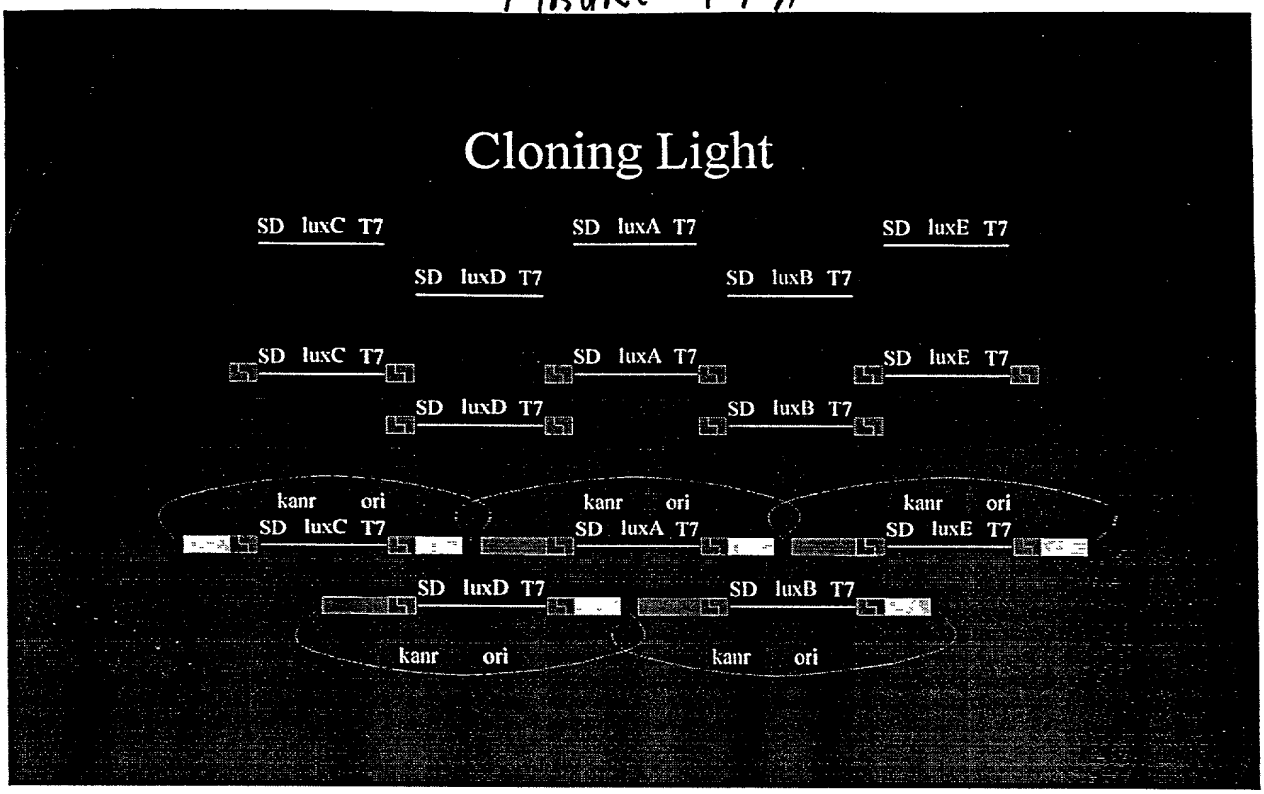


FIGURE 17B

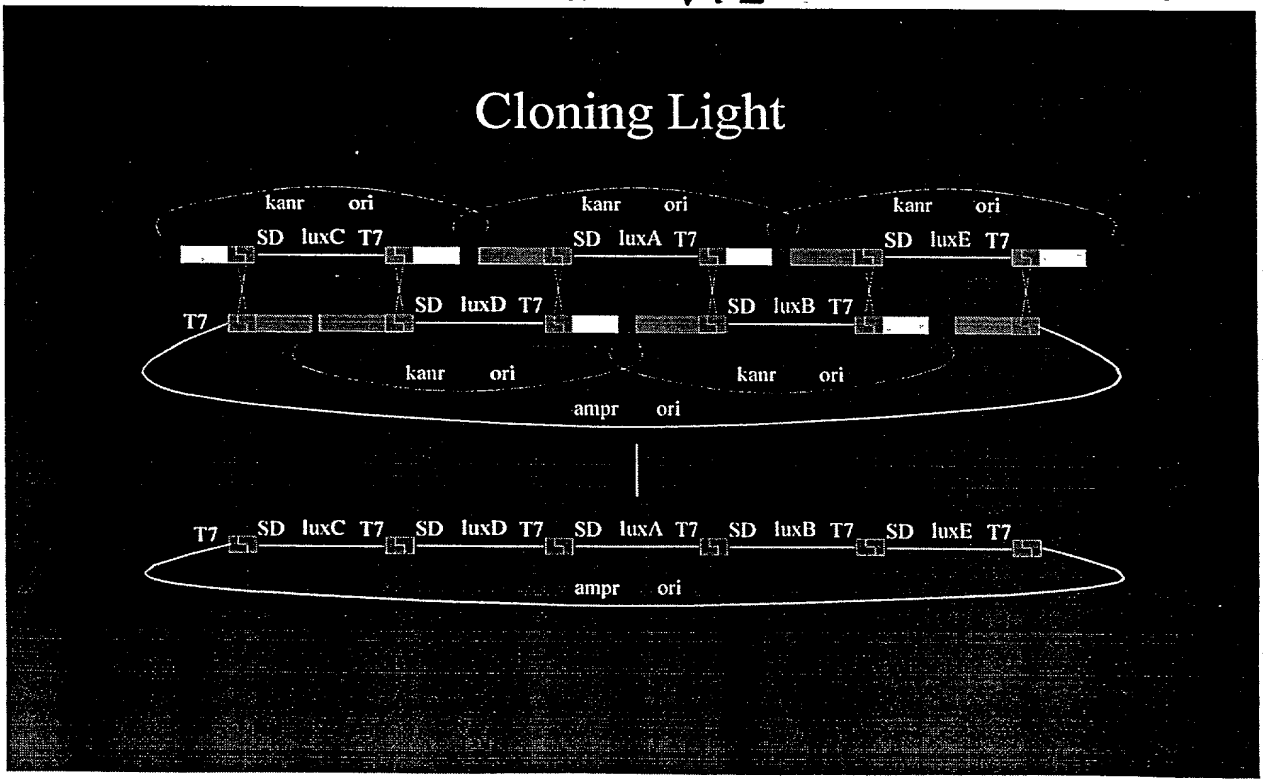


FIGURE 18

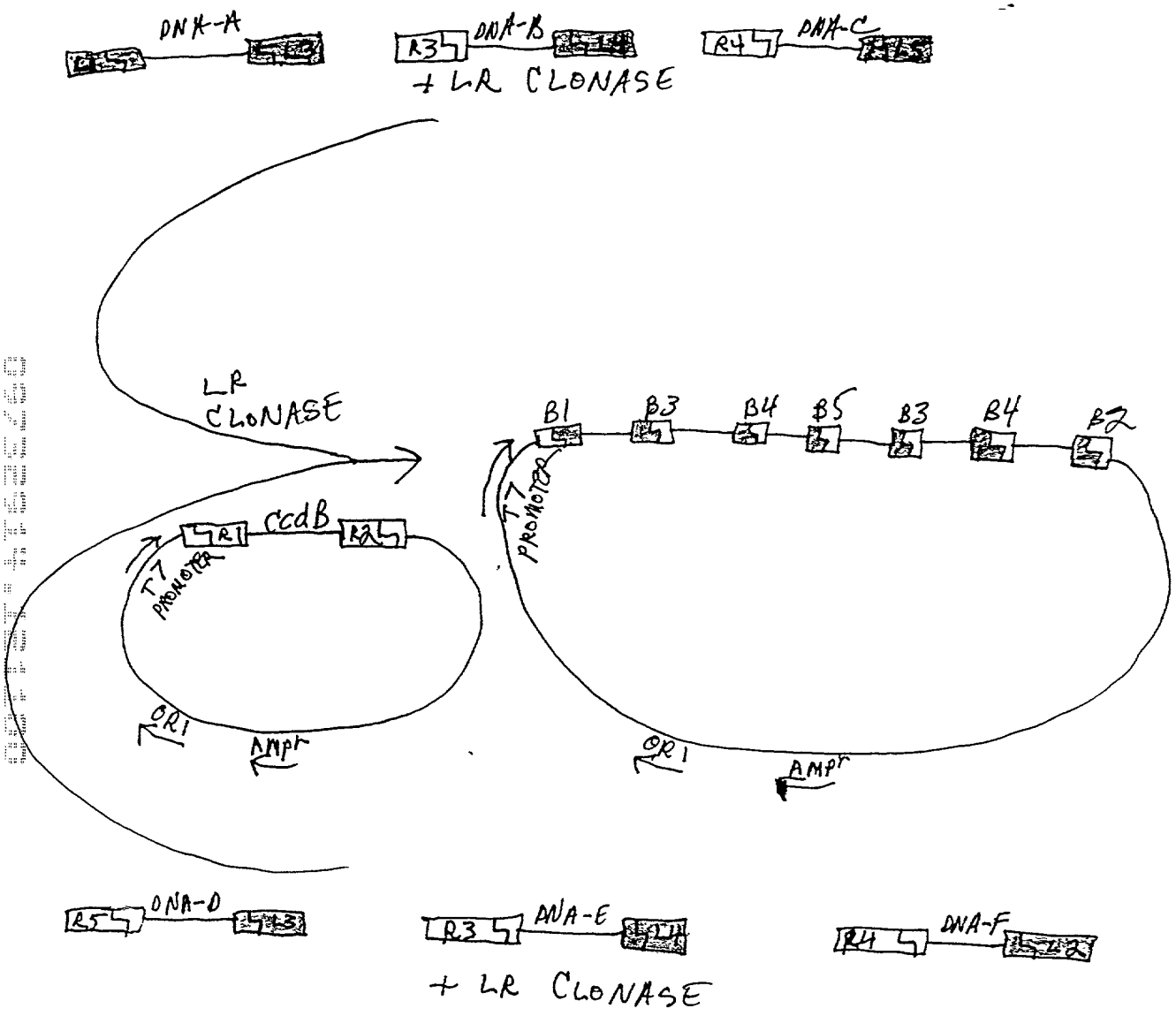

DNA-A
DNA-B
DNA-C
+ LR CLONASE
LR CLONASE
B1
B3
B4
B5
B3
B4
B2
T7 PROMOTER
R1
ccdB
R2
ORI
AMPr
DNA-D
DNA-E
DNA-F
+ LR CLONASE

FIGURE 19

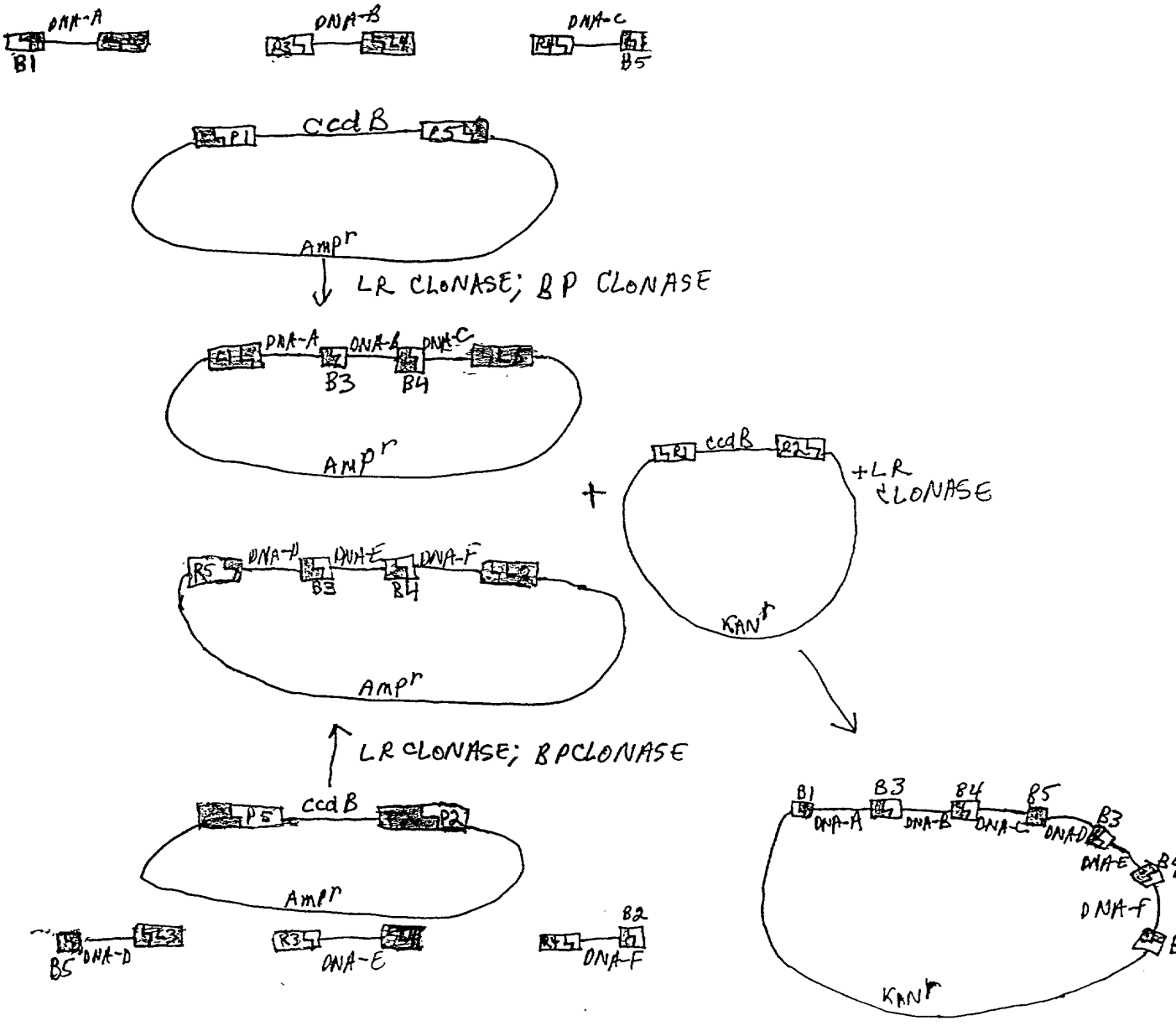

DNA-A
B1
DNA-B
DNA-C
B5
ccdB
P1
P5
Amp^r
LR CLONASE; BP CLONASE
DNA-A
DNA-B
DNA-C
B3
B4
Amp^r
+
ccdB
P2
+LR CLONASE
KAN^r
DNA-D
DNA-E
DNA-F
B3
B4
Amp^r
LR CLONASE; BP CLONASE
ccdB
P5
P2
Amp^r
B5
DNA-D
R3
DNA-E
B2
DNA-F
B1
B3
B4
B5
DNA-A
DNA-B
DNA-C
DNA-D
DNA-E
DNA-F
KAN^r

FIGURE 20

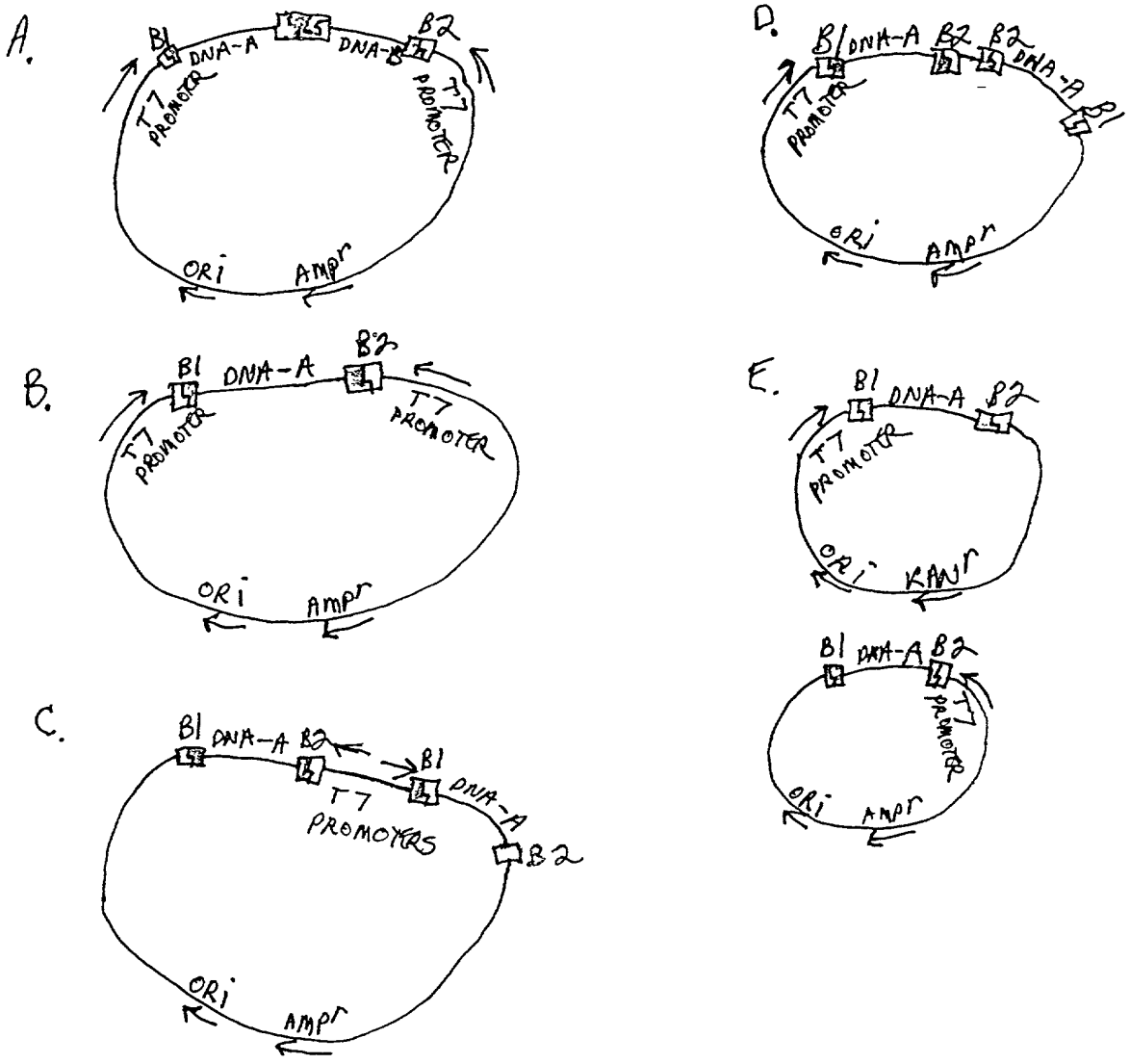

A.
B1
DNA-A
DNA-B
B2
T7 PROMOTER
T7 PROMOTER
ORi
AMPr
B.
B1
DNA-A
B2
T7 PROMOTER
T7 PROMOTER
ORi
AMPr
C.
B1
DNA-A
B2
B1
DNA-A
T7 PROMOTERS
B2
ORi
AMPr
D.
B1
DNA-A
B2
B2
DNA-A
B1
T7 PROMOTER
ORi
AMPr
E.
B1
DNA-A
B2
T7 PROMOTER
ORi
KANr
B1
DNA-A
B2
T7 PROMOTER
ORi
AMPr

FIGURE 21

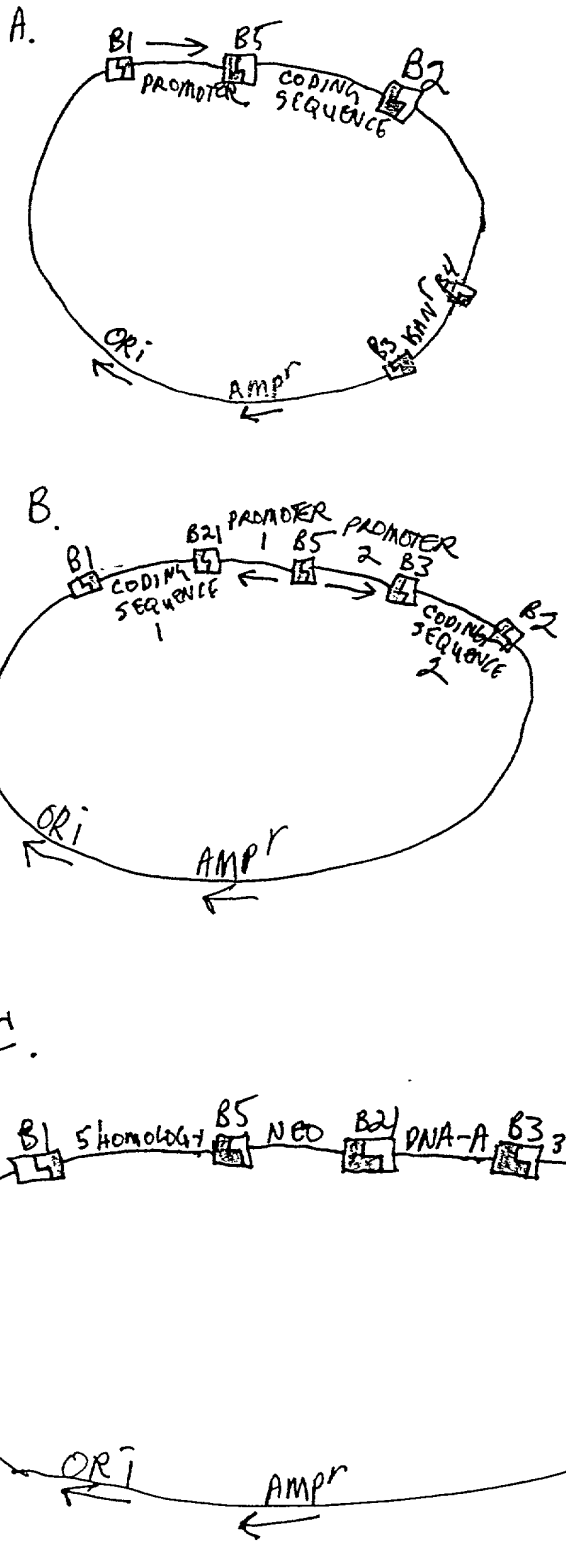
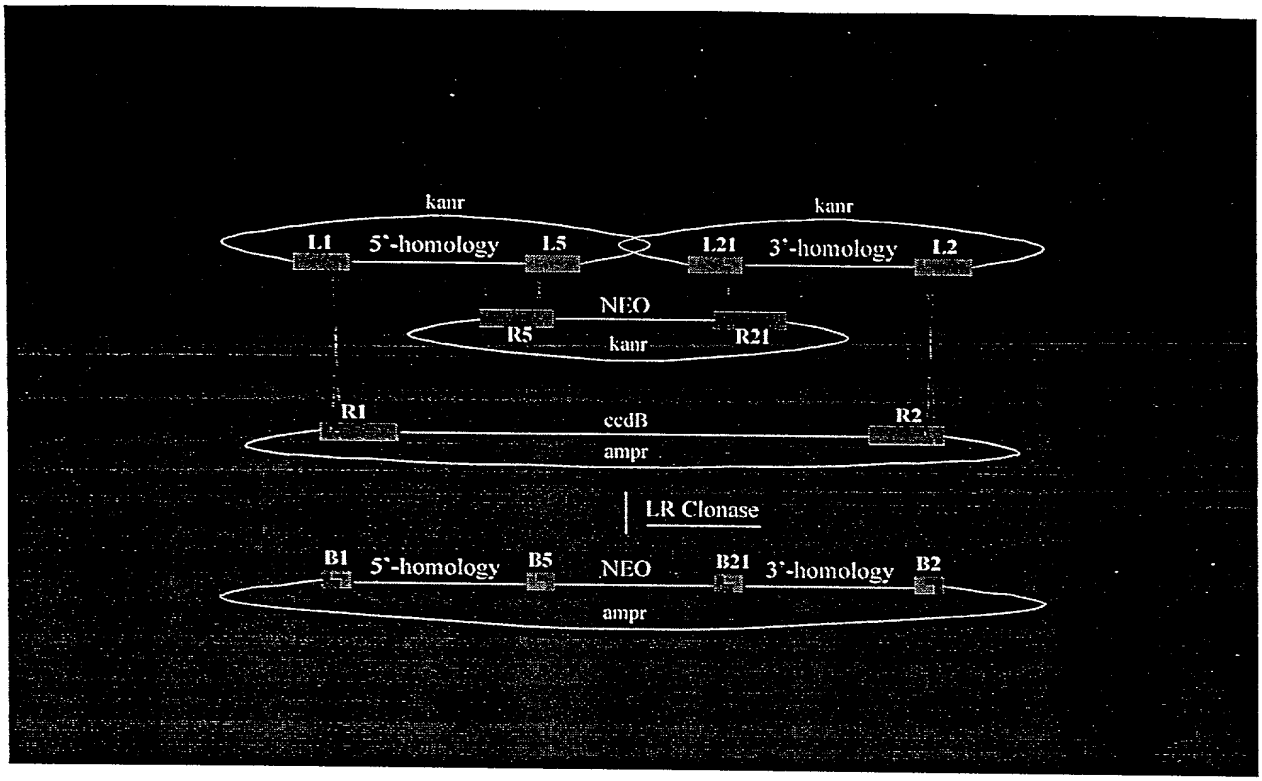

A.
B1
B5
PROMOTER
CODING SEQUENCE
B2
B3
KAN^r
ORi
Amp^r
B.
B1
B21
PROMOTER 1
B5
PROMOTER 2
B3
CODING SEQUENCE 1
CODING SEQUENCE 2
B2
ORi
Amp^r
C.
B1
5' Homology
B5
NEO
B21
DNA-A
B3
3' Homology
B2
ORi
Amp^r

FIGURE 22A



FIGUR 22 B

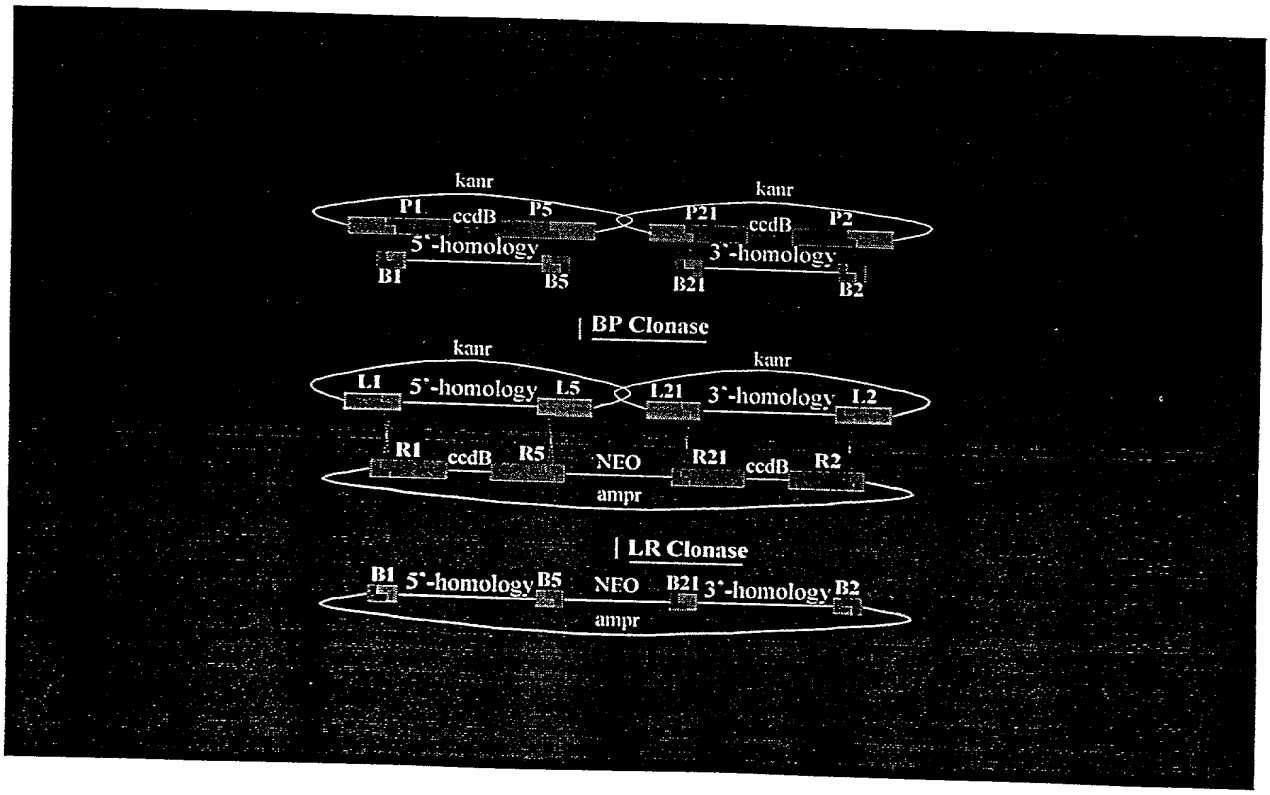


FIGURE 23

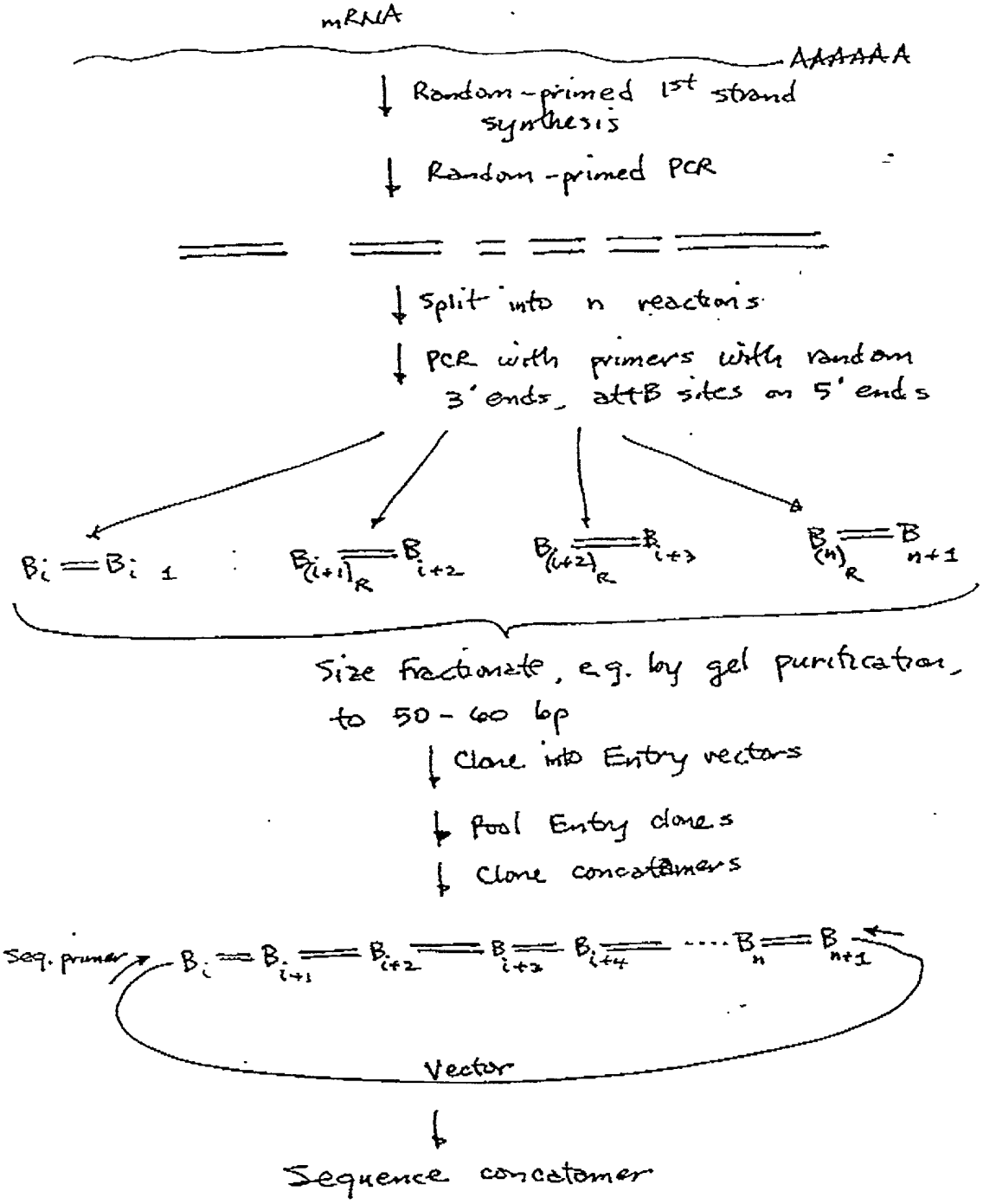

mRNA
AAAAAA
Random-primed 1st strand synthesis
Random-primed PCR
Split into n reactions
PCR with primers with random 3' ends, attB sites on 5' ends
Size fractionate, e.g. by gel purification, to 50 - 60 bp
Clone into Entry vectors
Pool Entry clones
Clone concatemers
Seq. primer
Vector
Sequence concatemer

FIGURE 24A

attB0 AGCCTGCTTT**TTTATAC**TAACTTGAGC (SEQ ID NO:1)
TCGGACGAAA**AAATATG**ATTGAACTCG

attP0 GTTCAGCTTT**TTTATAC**TAAGTTGGCA (SEQ ID NO:2)
CAAGTCGAAA**AAATATG**ATTCAACCGT

attL0 AGCCTGCTTT**TTTATAC**TAAGTTGGCA (SEQ ID NO:3)
TCGGACGAAA**AAATATG**ATTCAACCGT

attR0 GTTCAGCTTT**TTTATAC**TAACTTGAGC (SEQ ID NO:4)
CAAGTCGAAA**AAATATG**ATTGAACTCG

---

attB1 AGCCTGCTTT**TTTGTAC**AAACTTGT (SEQ ID NO:5)
TCGGACGAAA**AAATATG**TTTGAACA

attP1 GTTCAGCTTT**TTTGTAC**AAAGTTGGCA (SEQ ID NO:6)
CAAGTCGAAA**AAACATG**TTTCAACCGT

attL1 AGCCTGCTTT**TTTGTAC**AAAGTTGGCA (SEQ ID NO:7)
TCGGACGAAA**AAACATG**TTTCAACCGT

attR1 GTTCAGCTTT**TTTGTAC**AAACTTGT (SEQ ID NO:8)
CAAGTCGAAA**AAACATG**TTTGAACA

---

attB2 ACCCAGCTTT**CTTGTAC**AAAGTGGT (SEQ ID NO:9)
TGGGTCGAAA**GAATATG**TTTCACCA

attP2 GTTCAGCTTT**CTTGTAC**AAAGTTGGCA (SEQ ID NO:10)
CAAGTCGAAA**GAACATG**TTTCAACCGT

attL2 ACCCAGCTTT**CTTGTAC**AAAGTTGGCA (SEQ ID NO:11)
TGGGTCGAAA**GAACATG**TTTCAACCGT

attR2 GTTCAGCTTT**CTTGTAC**AAAGTGGT (SEQ ID NO:12)
CAAGTCGAAA**GAACATG**TTTGACCA

---

attB5 CAACTTT**ATTATAC**AAAGTTGT (SEQ ID NO:13)
GTTGAAA**TAATATG**TTTCAACA

attP5 GTTCAACTTT**ATTATAC**AAAGTTGGCA (SEQ ID NO:14)
CAAGTTGAAA**TAATATG**TTTCAACCGT

FIGURE 24B

*att*L5 CAACTTT**ATTATAC**AAAGTTGGCA (SEQ ID NO:15)
GTTGAAA**TAATATG**TTTCAACCGT

*att*R5 GTTCAACTTT**ATTATAC**AAAGTTGT (SEQ ID NO:16)
CAAGTTGAAA**TAATATG**TTTCAACA

---

*att*B11 CAACTTT**TCTATAC**AAAGTTGT (SEQ ID NO:17)
GTTGAAA**AGATATG**TTTCAACA

*att*P11 GTTCAACTTT**TCTATAC**AAAGTTGGCA (SEQ ID NO:18)
CAAGTTGAAA**AGATATG**TTTCAACCGT

*att*L11 CAACTTT**TCTATAC**AAAGTTGGCA (SEQ ID NO:19)
GTTGAAA**AGATATG**TTTCAACCGT

*att*R11 GTTCAACTTT**TCTATAC**AAAGTTGT (SEQ ID NO:20)
CAAGTTGAAA**AGATATG**TTTCAACA

---

*att*B17 CAACTTT**TGTATAC**AAAGTTGT (SEQ ID NO:21)
GTTGAAA**ACATATG**TTTCAACA

*att*P17 GTTCAACTTT**TGTATAC**AAAGTTGGCA (SEQ ID NO:22)
CAAGTTGAAA**ACATATG**TTTCAACCGT

*att*L17 CAACTTT**TGTATAC**AAAGTTGGCA (SEQ ID NO:23)
GTTGAAA**ACATATG**TTTCAACCGT

*att*R17 GTTCAACTTT**TGTATAC**AAAGTTGT (SEQ ID NO:24)
CAAGTTGAAA**ACATATG**TTTCAACA

---

*att*B19 CAACTTT**TTCGTAC**AAAGTTGT (SEQ ID NO:25)
GTTGAAA**AAGCATG**TTTCAACA

*att*P19 GTTCAACTTT**TTCGTAC**AAAGTTGGCA (SEQ ID NO:26)
CAAGTTGAAA**AAGCATG**TTTCAACCGT

*att*L19 CAACTTT**TTCGTAC**AAAGTTGGCA (SEQ ID NO:27)
GTTGAAA**AAGCATG**TTTCAACCGT

*att*R19 GTTCAACTTT**TTCGTAC**AAAGTTGT (SEQ ID NO:28)
CAAGTTGAAA**AAGCATG**TTTCAACA

FIGURE 24C

*att*B20 CAACTTT**TTGGTAC**AAAGTTGT (SEQ ID NO:29)
GTTGAAA**AACCATG**TTTCAACA

*att*P20 GTTCAACTTT**TTGGTAC**AAAGTTGGCA (SEQ ID NO:30)
CAAGTTGAAA**AACCATG**TTTCAACCGT

*att*L20 CAACTTT**TTGGTAC**AAAGTTGGCA (SEQ ID NO:31)
GTTGAAA**AACCATG**TTTCAACCGT

*att*R20 GTTCAACTTT**TTGGTAC**AAAGTTGT (SEQ ID NO:32)
CAAGTTGAAA**AACCATG**TTTCAACA

---

*att*B21 CAACTTT**TTAATAC**AAAGTTGT (SEQ ID NO:33)
GTTGAAA**AATTATG**TTTCAACA

*att*P21 GTTCAACTTT**TTAATAC**AAAGTTGGCA (SEQ ID NO:34)
CAAGTTGAAA**AATTATG**TTTCAACCGT

*att*L21 CAACTTT**TTAATAC**AAAGTTGGCA (SEQ ID NO:35)
GTTGAAA**AATTATG**TTTCAACCGT

*att*R21 GTTCAACTTT**TTAATAC**AAAGTTGT (SEQ ID NO:36)
CAAGTTGAAA**AATTATG**TTTCAACA

FIGURE 25A

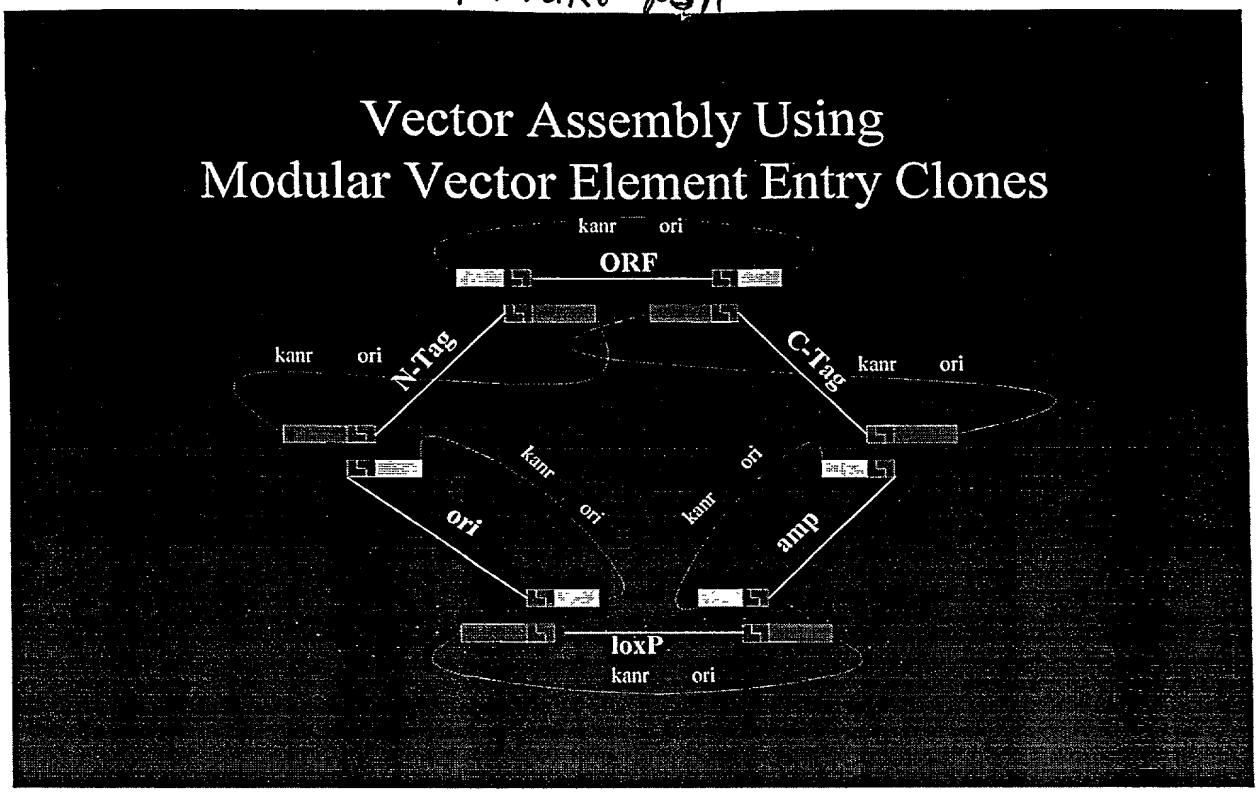


FIGURE 25B

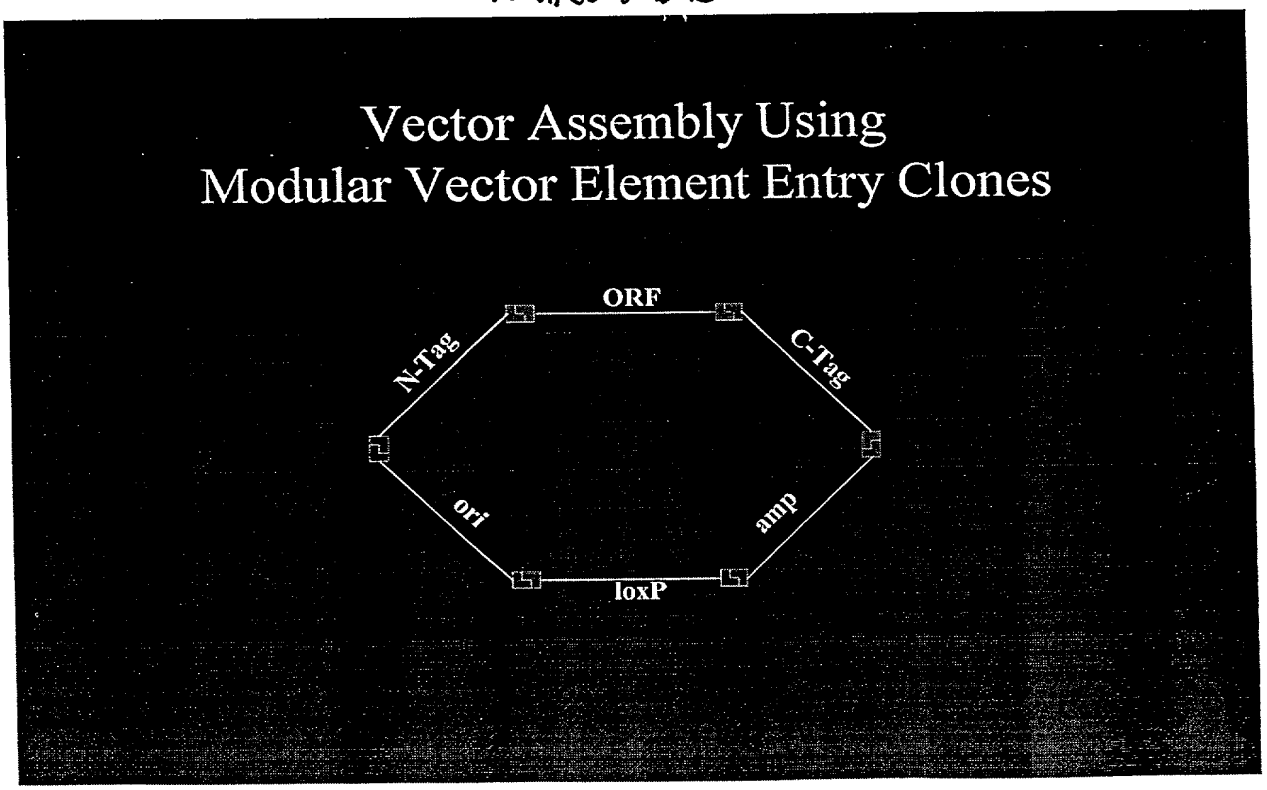


FIGURE 26A

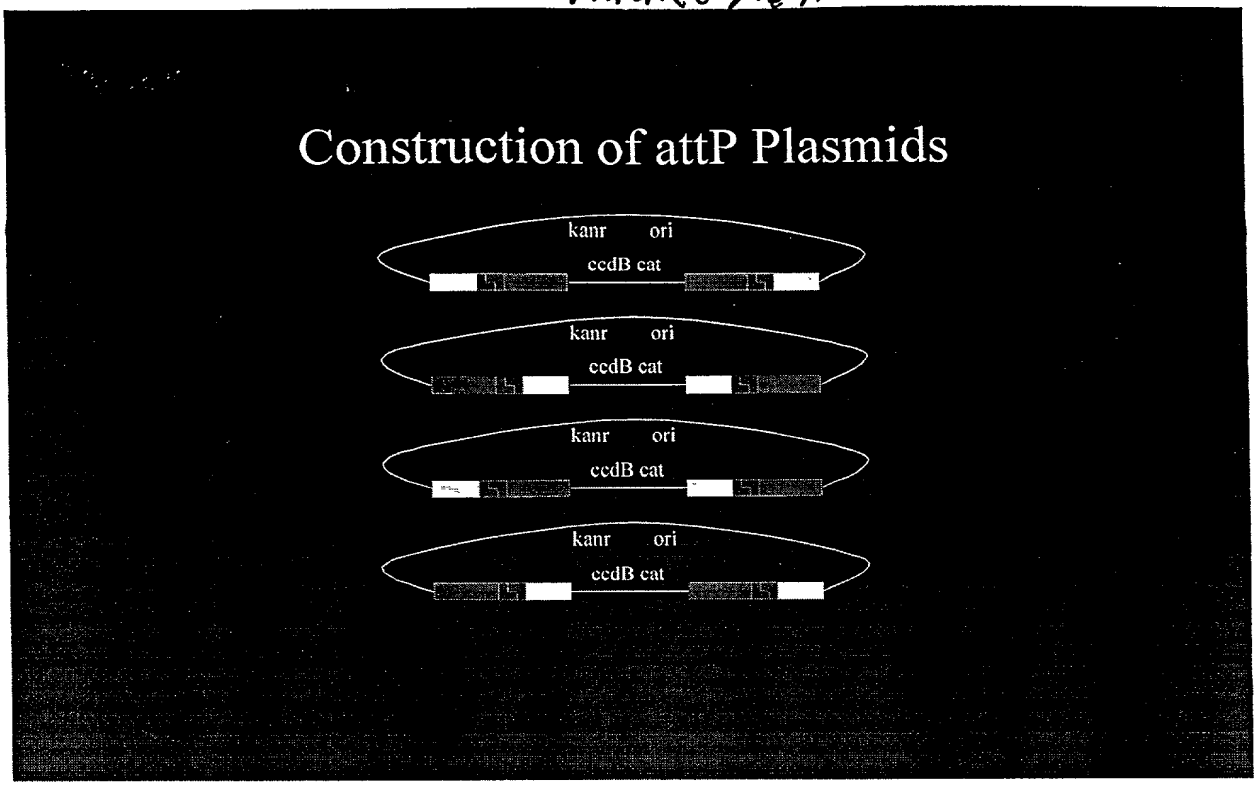
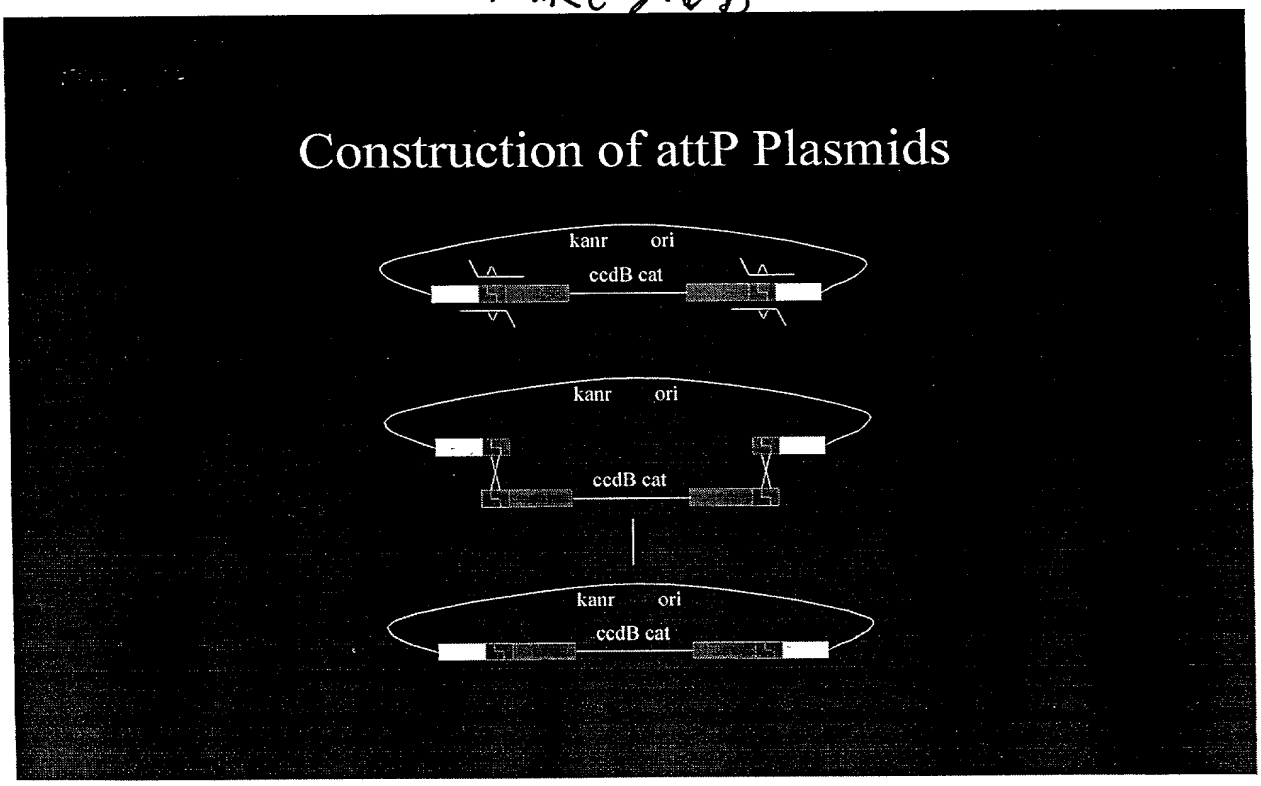
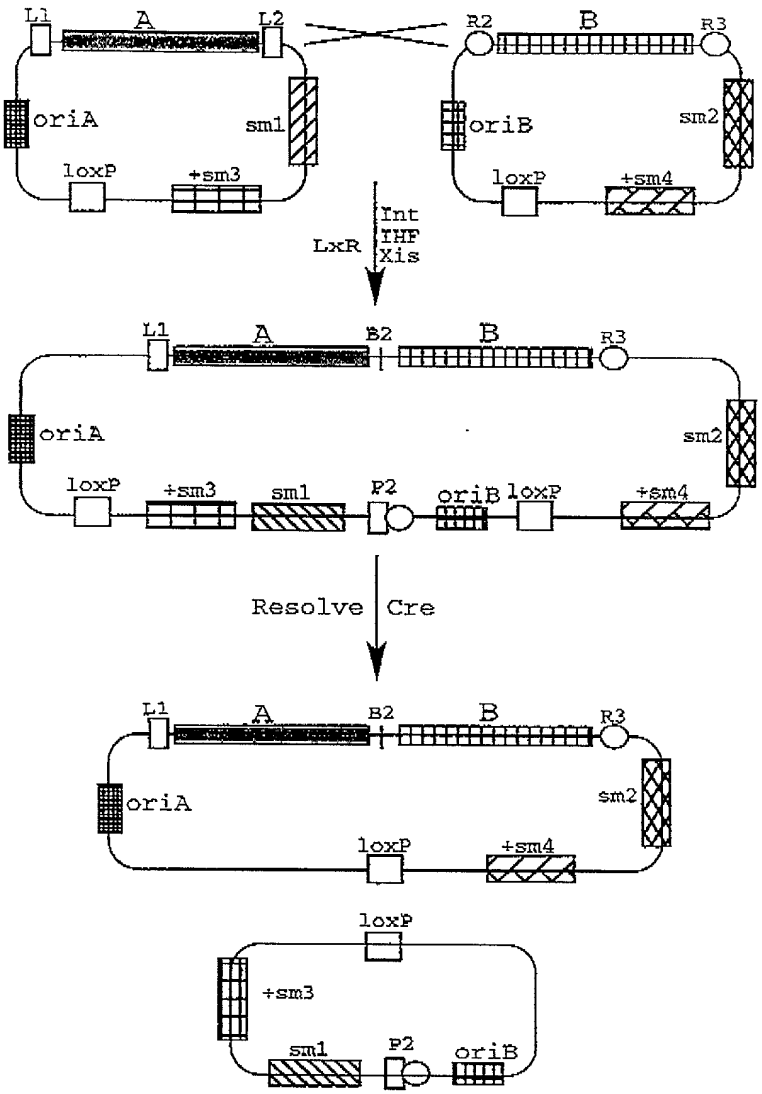


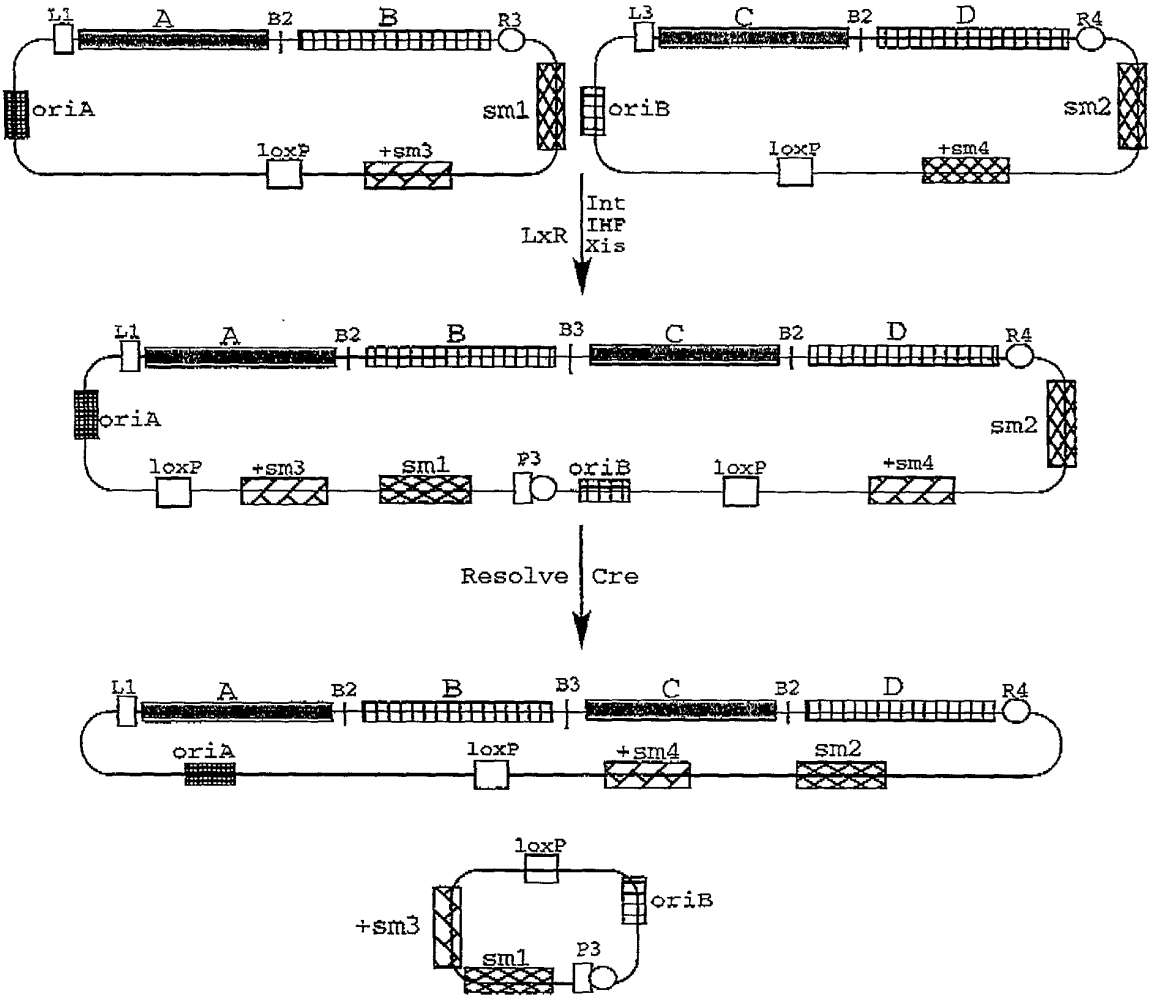
FIGURE 26B





Transform host that will support replication of oriA but not oriB and moreover, is sensitive to +sm3 but resistant to +sm4.

FIGURE 27A



Transform host that will support replication of oriA but not oriB and moreover, is sensitive to +sm3 but resistant to +sm4.

FIGURE 27B